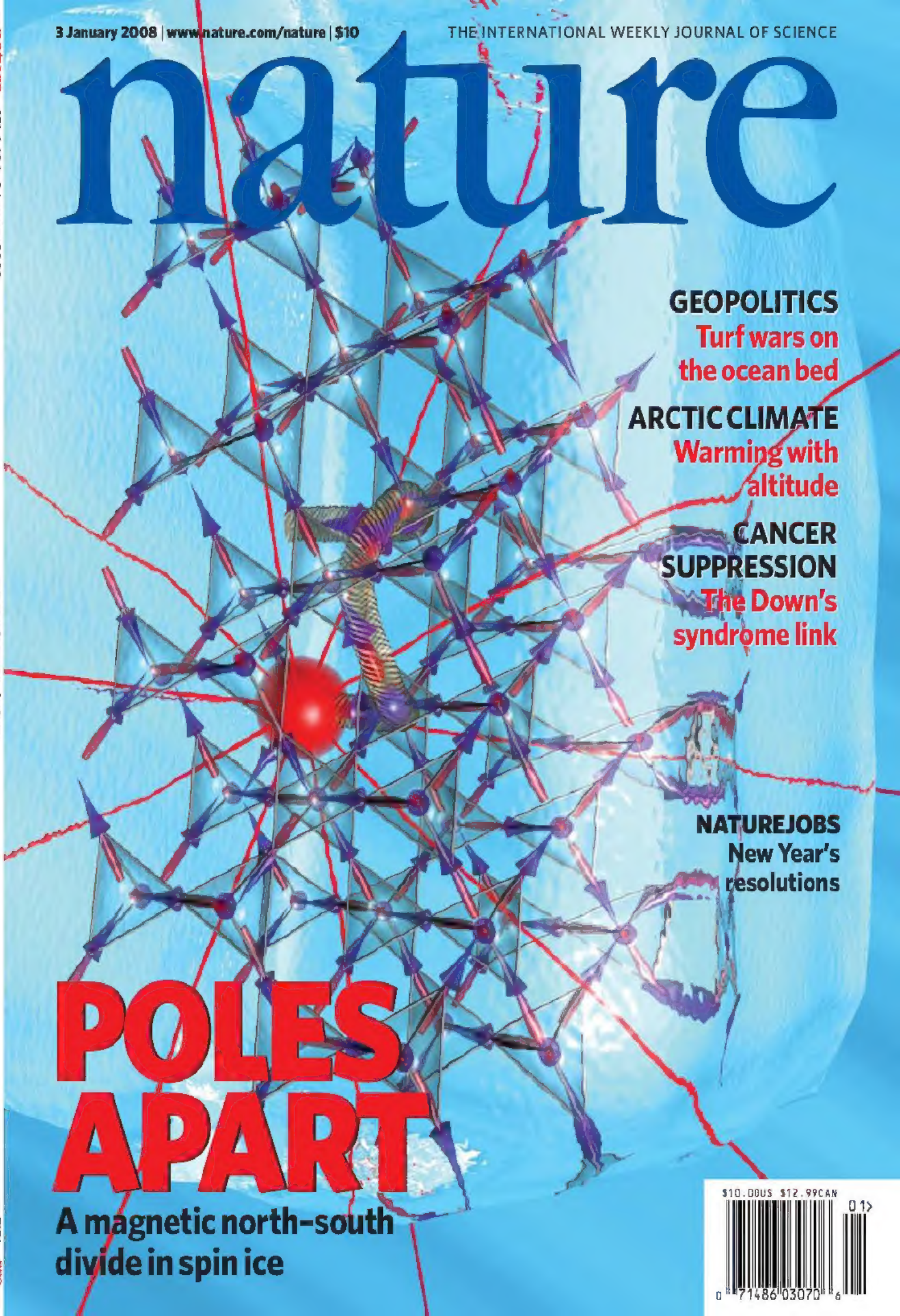
3 January 2008 | www.nature.com/nature | $10

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature



GEOPOLITICS
Turf wars on the ocean bed

ARCTIC CLIMATE
Warming with altitude

CANCER SUPPRESSION
The Down's syndrome link

NATUREJOBS
New Year's resolutions

POLES APART

A magnetic north–south divide in spin ice

$10.00US $12.99CAN

01>

0 71486 03070 6

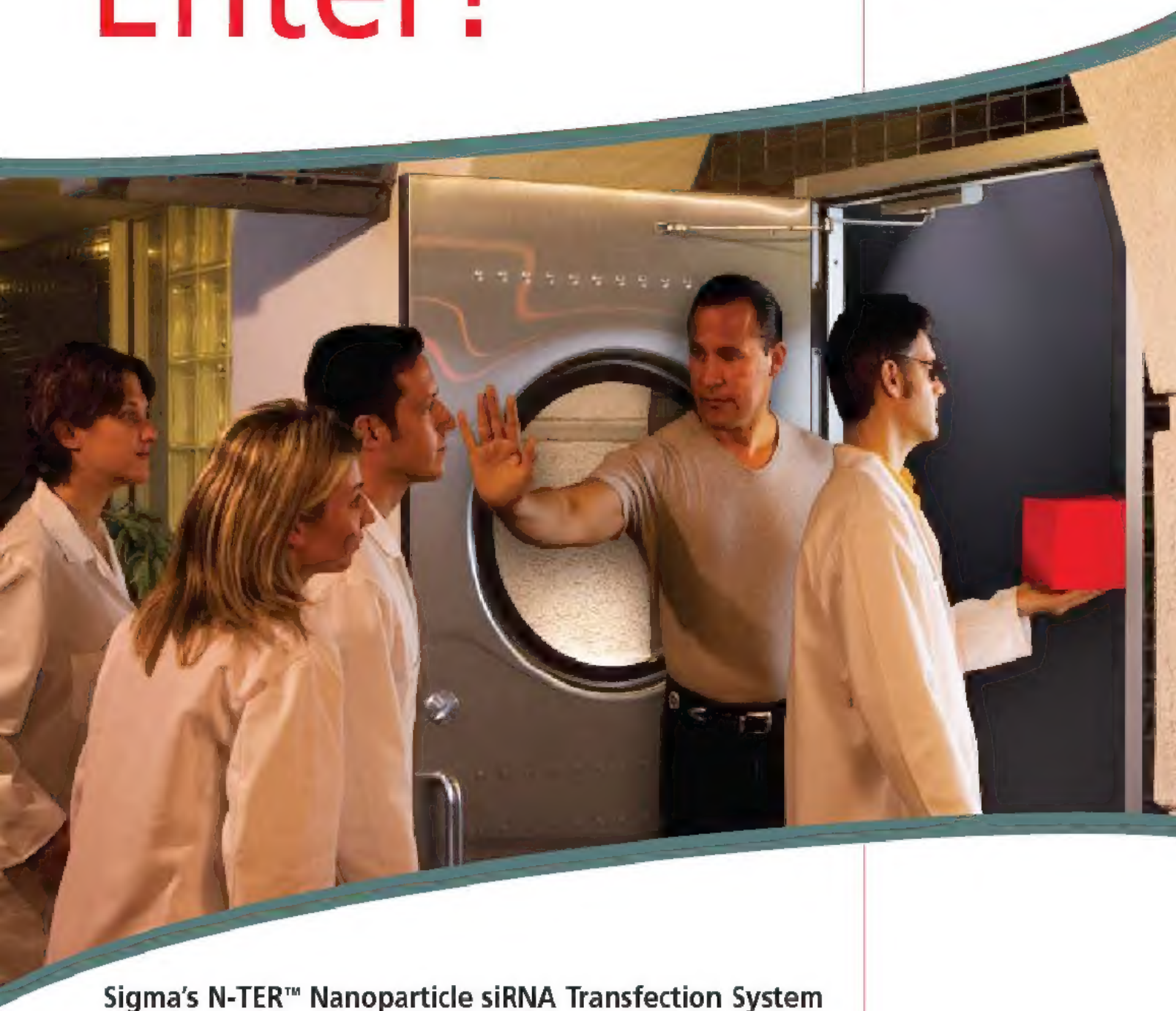
nature 451, 1-106 3 January 2008

www.nature.com/nature

no.7174 npg

Enter!

SIGMA Life Science



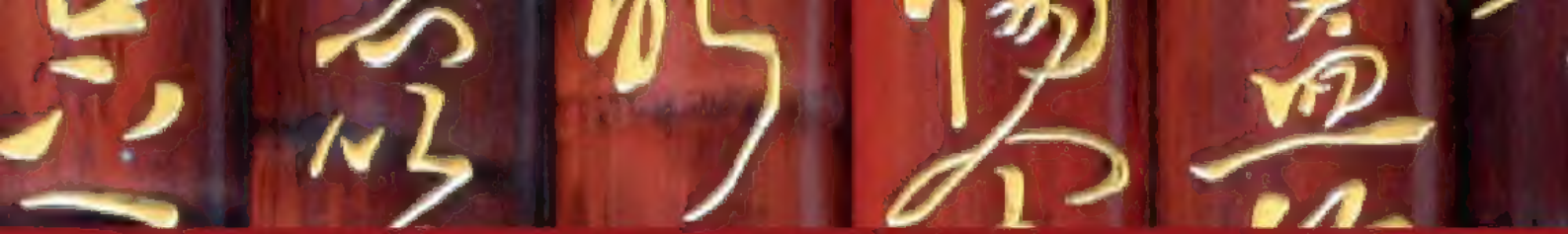
Sigma's N-TER™ Nanoparticle siRNA Transfection System gives you VIP access to difficult-to-transfect cells

- Simple transfection of primary, neuronal, differentiated, and non-dividing cells
- Quick delivery of siRNA into cells, with reduced cytotoxicity
- Stable N-TER/siRNA nanoparticle stocks can be stored and used for subsequent transfections
- Easily adapted protocol for high-throughput and reverse transfection uses

To learn more about how N-TER will open doors for you, visit *sigma.com/access*

Our Innovation, Your Research — Shaping the Future of Life Science

N-TER is a trademark of Sigma-Aldrich Biotechnology, L.P.



Sign up for *Nature China* table of contents e-alerts

Keep up to date with the latest leading research from Mainland China and Hong Kong by registering for *Nature China* e-alerts. Providing you with weekly research highlights from the region drawn from a variety of publications covering topics including:

- biotechnology
- cell & molecular biology
- chemistry
- clinical medicine
- developmental biology
- earth & environment
- ecology & environment
- genetics
- materials
- neuroscience
- physics
- space & astronomy

Go to **www.naturechina.com** and click on the e-alert sign up button for further details

For scientists in Mainland China and Hong Kong please visit: **www.naturechina.com.cn**

E-alert sign up

Supported by

AstraZeneca 阿斯利康

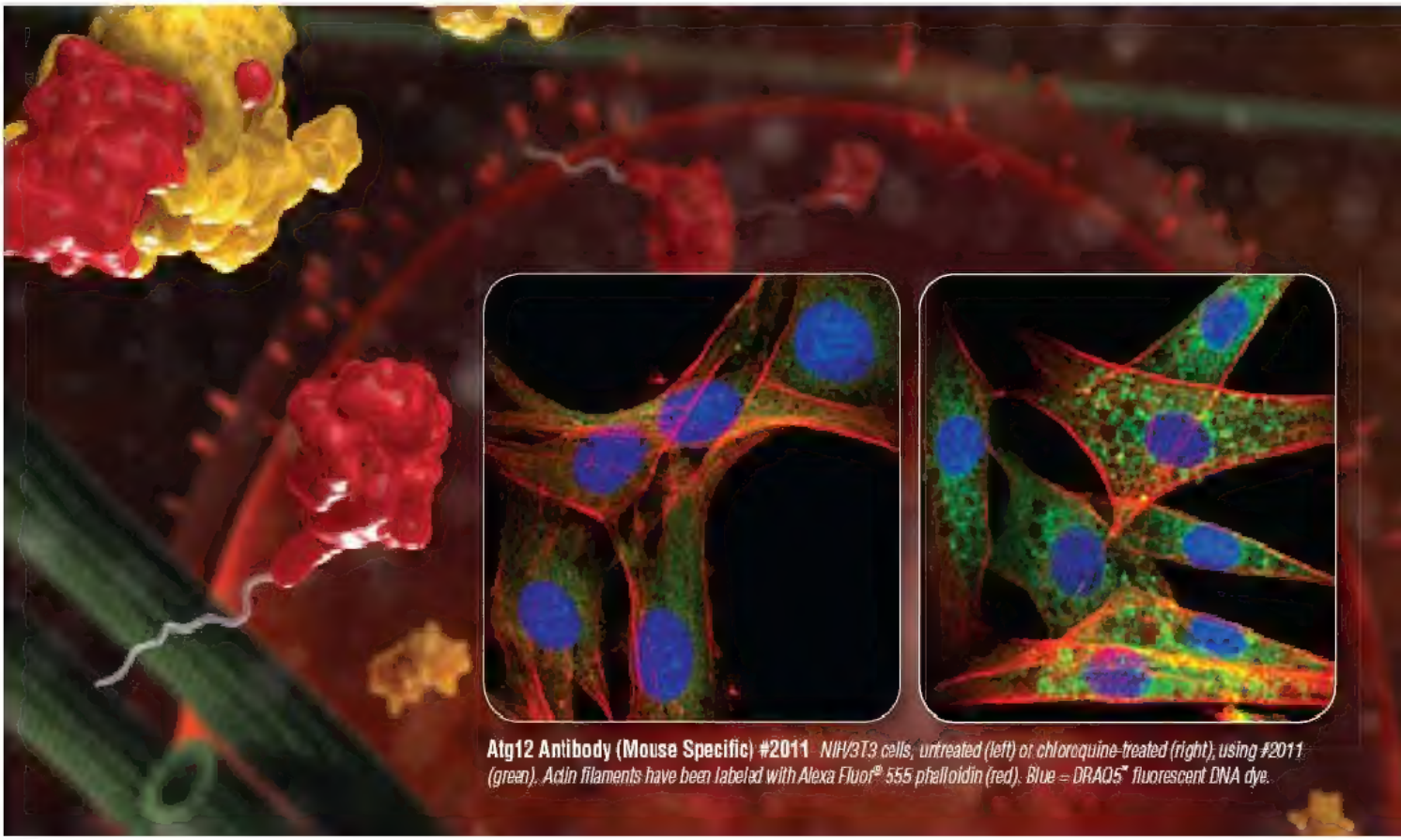
www.naturechina.com

nature publishing group npg

Innovative Discovery Tools for Signal Transduction Research

Antibodies for the Study of Autophagy

...from Cell Signaling Technology



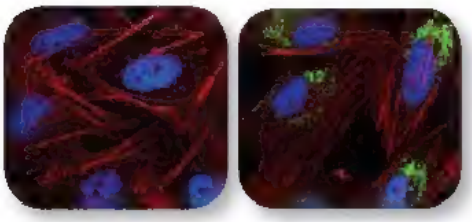
Atg12 Antibody (Mouse Specific) #2011 *NIH/3T3 cells, untreated (left) or chloroquine-treated (right), using #2011 (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue = DRAQ5™ fluorescent DNA dye.*

Unparalleled product quality, validation and technical support.

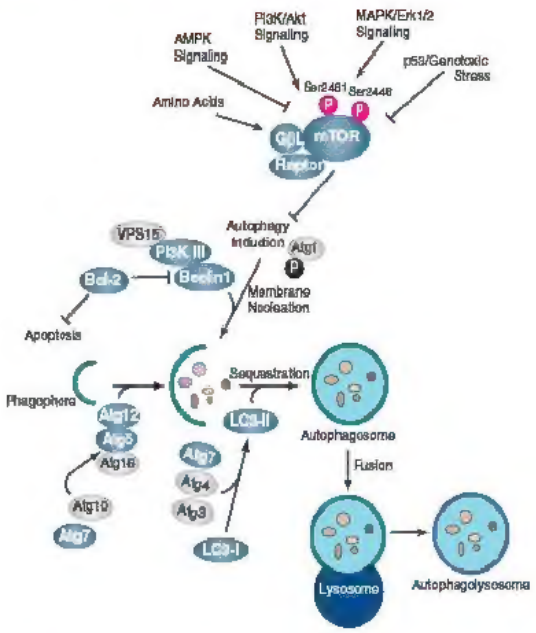
:: The highest quality antibodies from Cell Signaling Technology means that the antibodies will work the first and each time in all specified applications.

:: Extensive validation by our in-house clinical applications group means that optimization is not left up to you, the user.

:: Technical support provided by the same scientists who produce and validate the products translates into a thorough, fast and accurate response.



LC3B Antibody #2775
HeLa cells, untreated (left) or chloroquine-treated (right), using #2775 (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue = DRAQ5™ fluorescent DNA dye.



For a complete product listing visit...

www.cellsignal.com

Cell Signaling TECHNOLOGY®

Orders (toll-free) 1-877-616-CELL (2355) orders@cellsignal.com Fax ordering 1-978-867-2488 Technical support (toll-free) 1-877-678-TECH (8324) support@cellsignal.com Inquiries info@cellsignal.com

Nature Publishing Group
The Macmillan Building,
4 Crinan St, London N1 9XW, UK
e-mail: nature@nature.com

NATURE'S MISSION, 1869:
'The objective which it is proposed to attain by this periodical may be broadly stated as follows. It is intended, first, to place before the general public the grand results of scientific work and scientific discovery; and to urge the claims of science to move to a more general recognition in education and in daily life...Secondly, to aid scientific men [sic] themselves, by giving early information of all advances made in any branch of natural knowledge throughout the world, and by affording them an opportunity of discussing the various scientific questions which arise from time to time.'

Nature's mission statement was updated in 2000:
www.nature.com/nature/about

Submissions and Guide to Authors:
www.nature.com/nature/authors

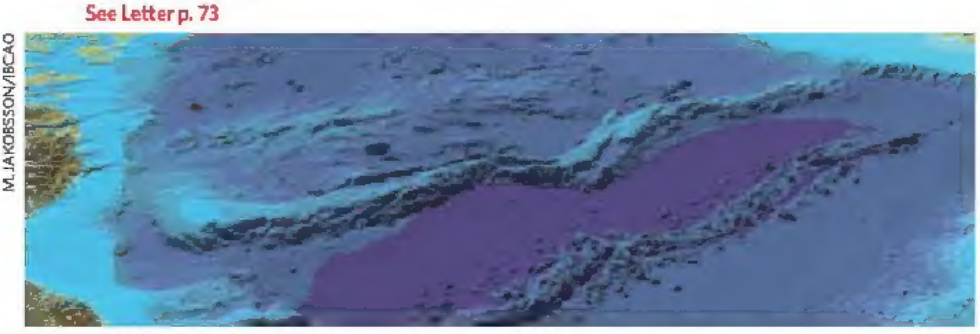
Author and referee policies and services:
www.nature.com/authors

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Nature Publishing Group, a division of Macmillan Publishers Ltd (The Macmillan Building, 4 Crinan Street, London N1 9XW). Registered as a newspaper at the British Post Office.
US Periodicals postage paid at New York, NY, and additional mailing post offices.
North and South American orders to: *Nature*, Subscription Dept, 342 Broadway PMB 301, New York NY 10013-3910, USA. Other orders to *Nature*, Brunel Road, Basingstoke, Hants RG21 2XS, UK.
Authorization to photocopy material for internal or personal use, or internal or personal use of specific clients, is granted by Nature to libraries and others registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided the relevant copyright fee is paid direct to CCC, 222 Rosewood Drive, Danvers, MA 01923, USA.
Identification code for *Nature*: 0028-0836/03.
US POSTMASTER: send address changes to *Nature*, Subscription Dept, 342 Broadway PMB 301, New York, NY 10013-3910, USA; CPC PUB AGREEMENT #40032744
Published in Japan by NPG Nature Asia-Pacific, Chiyoda Building, 2-37 Ichigayatamachi, Shinjuku-ku, Tokyo 162-0843, Japan.
© 2008 Nature Publishing Group

nature publishing group

nature

High profile: a celeb in the lab? Futures, p. 106.



Disputed territory: the Lomonosov ridge is seen as a passport to oil riches. News Feature, p. 12.

Gene Knockdown guaranteed.

HuSH-29™ shRNA Expression Plasmids

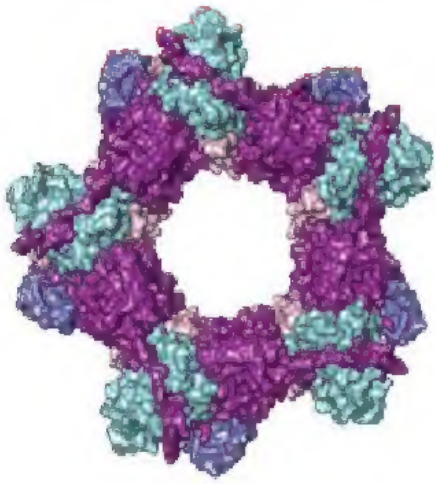
- All shRNA vectors delivered as 5 ug of highly purified, transfection-ready plasmid DNA. No plasmid preparation required.
- The successful use of OriGene shRNA vectors have been recently cited
- OriGene shRNAs can be rapidly validated in HEK293T cells together with OriGene TrueClone cDNAs

Downregulation of HIF1A Expression by HuSH Contructs

HEK239T cells were contransfected with pCMV-HIF1A cDNA together with four shRNA contructs against HIF1A. Western blot data demonstrates that three out of the four constructs significantly downregulates the cotransfected HIF1A expression.
More details can be found at www.origene.com/rna

ORIGENE
Your Gene Company

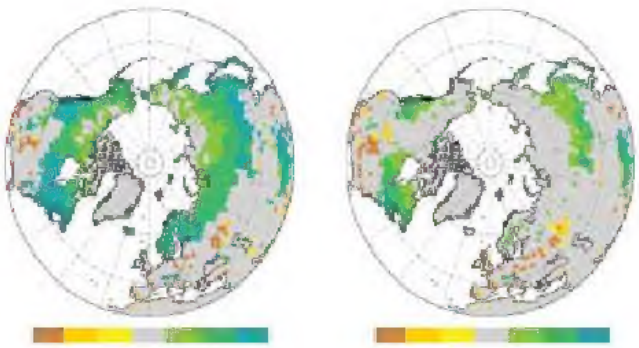
1-888-267-4436 • origene.com



The molecules behind hydrogen peroxide-mediated cell signalling, p. 98.

ARTICLES

LETTERS



Riding for a fall: autumn warming in the north may hit carbon sinks, pp. 49, 26.

NATURE ONLINE

ADVANCE ONLINE PUBLICATION

PUBLISHED ON 2 JANUARY 2008

Predicting expression patterns from regulatory sequence in *Drosophila* segmentation

E Segal, T Raveh-Sadka, M Schroeder, U Unnerstall & U Gaul

doi:10.1038/nature06496

PODCAST NEWS

Subscribe via iTunes or go to the link below to download the latest Nature Podcast. Out every Thursday, the free podcast adds background to *Nature* papers and features with author interviews and scientific discussions. 'Back issues' can be downloaded from the archive on http://tinyurl.com/k62c.

www.nature.com/podcast

THIS ISSUE

FLYING THE FLAG The recent flag-planting photo opportunity involving a Russian submersible on the Lomonosov ridge beneath the Arctic Ocean is the most dramatic example yet of a growing trend.

RTR RUSSIAN CHANNEL/AP

Deep thought: floating the flag.

Geophysicists are being recruited to back up national claims on the seafloor and its associated mineral wealth. Daniel Cressey reports on the politicization of a science, and the legal wrangling that we can anticipate in the years to come as competing claims are considered. [News Feature p. 12]

WORLDS VIEWED Dimitar Sasselov answers the questions you meant to ask about extrasolar planets. How many are there? How do we know? Do they contain water? And of course, the big question: could we live on one of them? [News & Views Feature p. 29] Elsewhere in the issue, on page 38, an affirmative answer to the question, 'Have they found a young extrasolar planet yet?'

RANK INSIDER The SCImago Journal & Country Rank, developed from the Google PageRank algorithm, is making waves in scientific circles. Its journal rankings differ from, and make interesting comparison with, those derived from impact factors. Declan Butler, *Nature*'s resident search-engine guru, keeps abreast of developments in this arcane field and writes this week on the current state of play. An alternative ranking system is welcome, it seems. But what they actually tell us is less clear. [See News]

A GOOD BOOK Evolutionary biology has seen some remarkable advances in the past 30 years, with new fields like 'evodevo' and evolutionary genomics expanding the scope of the field in all directions. This means there is always room for a good up-to-date textbook. Our reviewer rates *Evolution*, from Cold Spring Harbor Laboratory, as one of the best. With support promised from http://www.evolution-textbook.org/, the book weighs in at a convenient size and scores well on readability. [Books & Arts p. 17]

We are familiar with elementary particles that carry either negative or positive electric charge, such as electrons and protons, but there is no evidence of elementary particles with a net magnetic charge. Magnets tend to come with inseparable north and south poles, and there are no known magnetic monopoles despite concerted efforts to find them. But an intriguing theoretical study now proposes that magnetic monopoles may exist, not as elementary particles, but as emergent particles in exotic condensed matter magnetic systems such as 'spin ice'. The theory, based on an analogy to fractional electric charges seen, for example, in quantum Hall systems in two dimensions, can explain a mysterious phase transition that has been observed experimentally in spin ice. The cover, by Alessandro Canossa, depicts a magnetic monopole (red sphere) emerging from break-up of the dipole moment (arrows) of the underlying electronic degrees of freedom in spin ice. [Letter p. 42; News & Views p. 22]

Warming with altitude

Some of the most pronounced signs of climate change have been seen in the Arctic, for example, near-surface warming there has been almost twice the global average over the past few decades. The underlying causes of this 'Arctic amplification' remain uncertain, but examination of a temperature data set based on modelling and observations in the region during this period provides some clues. The key finding is evidence for atmospheric temperature amplification well above the surface. This is unlikely to be a due to reduced snow and ice cover during the greater part of the year, suggesting that factors such as changes in atmospheric heat transport may be involved in the recent Arctic warming. [Letter p. 53]

Down's syndrome cancers

Some epidemiological studies have suggested that individuals with Down's syndrome (who carry three copies of chromosome 21, known as trisomy 21) show a reduced incidence of solid tumours. Other studies failed to confirm this. Experiments in the Ts65Dn mouse model of Down's syndrome, trisomic for about 100 genes, may have resolved these contradictory findings. They reveal that trisomy for a subset of mouse equivalents of chromosome 21 genes reduces the incidence of some intestinal tumours, yet the presence of one copy of the same genes increases the number of tumours. The dosage-dependent effect is attributed to the Ets2 transcription factor. So *Ets2*, known until now as an oncogene, is also a tumour repressor, and is a potential target for anti-cancer prophylaxis. [Letter p. 73; News & Views p. 21]

Life after sex

Most insect females undergo a profound switch in reproductive behaviour once they mate: they become unreceptive to courting males and start laying eggs. Mosquito females, in particular, start seeking a blood meal after sex. This transformation is triggered by factors present in the male seminal fluid, and in 1988 the active factor in *Drosophila* was found to be a small peptide, dubbed the 'sex peptide'. Now the long-sought receptor protein for this peptide has been identified. The sex peptide

Settling down: egg-laying is just one consequence of sex.

receptor — which turns out to be the orphan receptor CG16752 — functions in a subset of neurons implicated in other sex-related behaviours. The receptor is highly conserved across insect species, raising the possibility that it could be targeted to disrupt reproduction in insect pests or host-seeking behaviour in disease vectors. [Article p. 33; News & Views p. 24]

A young exoplanet at last

The number of known extrasolar planets is well into three figures, but until now, none has been what you might call 'young'. Now a massive young planet, six times the mass of Jupiter, has been detected in the dust disk around

the nearby star TW Hydrae (TW Hya). At just 8–10 million years old, TW Hya had already been identified as a likely location for developing planets, and the building blocks for a planetary system are present in its circumstellar disk. The new observation is significant as it puts a direct constraint on the timescale of extrasolar planet formation. [Letter p. 38]

Nanocluster structures

It is difficult to determine the three-dimensional structure of ultrasmall nanoparticles as they are unstable and tend to interact with any incident electron beams used to examine them. Now, using aberration-corrected scanning transmission electron microscopy coupled with imaging simulation, the size, shape, orientation and atomic arrangement of specially prepared size-selected gold nanoclusters have been determined to single-atom resolution. The particles were preformed in the gas phase and soft-landed on an amorphous carbon substrate. These materials are of interest for catalytic and biological applications. [Letter p. 46]

Autumn warming

An analysis of variations in atmospheric CO_2 and ecosystem CO_2 fluxes in the Northern Hemisphere shows that warmer autumns have been associated with an earlier autumn-to-winter CO_2 build-up in the atmosphere. This seems counter-intuitive: warm autumns surely imply long growing seasons and a beneficial effect on terrestrial carbon sinks as trees and plants make more biomass. An explanation is provided by satellite observations and numerical modelling. Enhanced respiration caused by higher temperatures causes carbon losses that offset photosynthetic gains, limiting the potential of these ecosystems to act as carbon sinks. And CO_2 loss due to autumn warming may offset most of the increased CO_2 uptake during spring. If future warming occurs more rapidly in autumn than in spring, the ability of northern ecosystems to sequester carbon may diminish more rapidly than previously predicted. [Letter p. 49; News & Views p. 26]

Earthquakes take the strain

Small strains induced by seismic waves can trigger earthquakes thousands of kilometres away, with failure often occurring long after the waves have passed. The mechanism behind this phenomenon of dynamic earthquake triggering is unknown. Lab studies of granular friction have become a useful tool for investigating fault zone processes, as shown by new experiments tracking stick–slip in granular media (glass beads), with acoustic waves used to simulate earthquake triggering. The results show that small magnitude failure events, corresponding to triggered aftershocks, occur when sound waves of sufficient amplitude are applied. Vibrations also cause large slip events to be disrupted in time relative to those without wave perturbation, suggesting that dynamic stressing of tectonic faults may play a role in determining the complexity of earthquake recurrence. [Letter p. 57]

NUMB's anticancer action

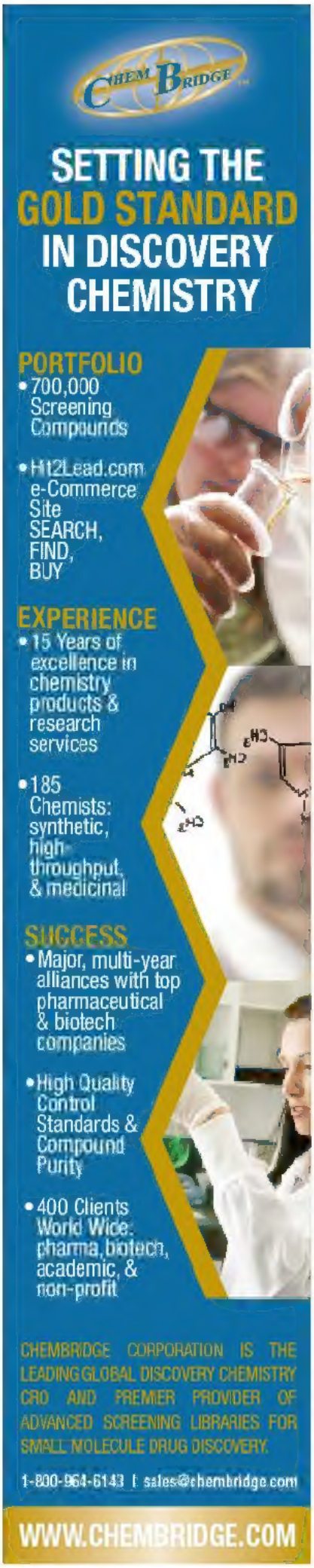
The NUMB protein is involved in cell fate decisions via an interaction with a NOTCH family plasma membrane receptor, and plays a role in endocytosis. Its expression was known to be downregulated in human breast cancers. Now NUMB has been found to act as a tumour suppressor protein by inhibiting the ubiquitin ligase HDM2, thereby preventing the destruction of the major tumour suppressor p53. In addition, low levels of NUMB expression in breast tumours are found to be associated with a poor prognosis. These findings connect two areas of cell biology previously considered unrelated, and are of potential relevance for the design of rational therapies for cancer. [Letter p. 76]

Aiming high in biofuels

'Higher' alcohols offer advantages over ethanol as biofuels thanks to their higher energy densities and lower hygroscopicities, and 'branched' alcohols have higher octane numbers than their straight-chain counterparts. But these other alcohols cannot be synthesized economically using native microorganisms. Now an *Escherichia coli* strain has been re-engineered to produce higher alcohols (including isobutanol, 1-butanol and 2-phenylethanol) from glucose, a renewable carbon source. The strategy involves diverting intermediates from the amino acid biosynthetic pathway to generate the desired alcohol and may facilitate large-scale production of biofuels by microbial fermentation. [Letter p. 86]

Old world order

Life on Earth is believed to have evolved from an 'RNA world' where RNA molecules both catalysed essential chemical reactions and carried genetic information. In modern biology, proteins have become the enzymatic workhorses of the cell, while nucleic acids retain the informational role. Within cells, however, relics of the RNA world remain. One such is the mitochondrial tyrosyl-tRNA synthetase, CYT-18, from the fungus *Neurospora crassa*, which also binds a group I intron ribozyme and facilitates splicing. The crystal structure of this protein/ribozyme complex has now been determined. The interaction surface is different from that used by CYT-18 to bind $tRNA^{Tyr}$ in its enzymic role. Specific changes are found which do not exist in non-splicing tRNA synthetases, suggesting ways in which RNA–protein complexes could have evolved from RNA-only enzymes. [Letter p. 94]



Abstractions

FIRST AUTHOR

Astronomers believe that planets form in 'protoplanetary disks' — swirling masses of gas, dust and other particles that surround newborn stars. But direct proof of this theory has been lacking, and the timescales over which planets form, as well as the process by which they do so, are still up for debate. On page 38, Johny Setiawan and his colleagues at the Max Planck Institute for Astronomy in Heidelberg, Germany, reveal their discovery of a giant planet orbiting a star young enough to still be surrounded by a protoplanetary disk. This is a key piece of evidence in the endeavour to understand planet formation.

Were you determined to prove that the protoplanetary disk is deservedly named?

Yes. By studying planet formation we hope to understand the origin of planetary systems and put our solar system in a universal context. To do so, we have to look among the more than 100 young stars with documented circumstellar disks, in which we believe planets are born. Previous work drew attention to TW Hydrae, an 8 million to 10 million-year-old star. There was speculation that variations in its disk structure could be due to a planet forming. So we decided to take a closer look.

Why has no one found this evidence before?

Previous work focused on the quickest way to discover extrasolar planets — using radial velocity, which measures changes in an object's velocity along the line of sight over time. Most researchers excluded young stars from such surveys because they are rife with noisy data resulting from stellar activity. Now that more than 270 extrasolar planets have been found, attention is turning to the physics of young stars to help us understand the birth of planetary systems. We used radial velocity to search young stars one by one and extracted information carefully. We were lucky that the planet we found is big enough for us to detect around a young star.

Do your findings change our understanding of planet formation?

Our work gives an observational upper limit for the timescale of giant planet formation. Statistical studies of young stars suggested that disk lifetime can be a few tens of millions of years. More recent studies put a typical disk lifetime at about 10 million years. Our work indicates that planet formation should be complete within 8 million years.

Do you intend to search for other planet-forming protoplanetary disks?

Yes. But we are also continuing to observe TW Hydrae. A companion planet could be forming in the disk around it. ■

MAKING THE PAPER

Roger Reeves

Down's syndrome holds genetic clue to cancer prevention.

Scientists have struggled for more than 50 years to resolve the controversial claim that individuals with Down's syndrome are less likely to develop solid tumours. Although the idea has become accepted dogma in recent years, studies hoping to prove or disprove the theory have been less than definitive. Reports of research showing cancer rates in people with Down's syndrome to be equal to or greater than those in the general population appear in the literature just as frequently as those concluding that rates are lower.

The difficulty of searching for low-frequency cancers in an already small sample size (only 1 in 700 people have the extra copy — known as 'trisomy' — of chromosome 21 that leads to Down's syndrome), confounds epidemiological studies. "Looking for lower incidence of an already very rare event makes it difficult to obtain an adequate sample size, which is the Achilles' heel in these studies," says Roger Reeves, a geneticist at the Johns Hopkins University School of Medicine in Baltimore, Maryland. In addition, he says, some studies make no adjustments for the generally shorter lifespan seen in Down's syndrome.

About five years ago, Reeves made what he calls a "leap of faith" after taking a good look at the conflicting epidemiological data. He decided that the statistics had reached an impasse and opted to take a biological approach based on mouse models of Down's syndrome. By studying mice with three copies of a group of mouse genes that correspond to a subset of genes found on human chromosome 21, Reeves and his colleagues have pin-pointed a dosage-dependent tumour 'repressor' gene that may hold promise for cancer prevention (see page 73).

Early in the study, the team showed that a genetic cross between trisomic mice and mice carrying a gene associated with a high proportion of intestinal cancers reduced tumour formation by almost half. Then, Reeves' doctoral student, Thomas Sussan, narrowed the search for the responsible genes by using a mutant mouse with fewer triplicate genes — just 33.

Having found that this also lowered tumour incidence, the team looked more closely at the subset of 33 genes. They found that, despite being known to cause cancer when mutated, in triplicate the transcription factor Ets2 decreases tumour incidence.

As he became more involved with individuals with Down's syndrome, Reeves uncovered much misinformation about their quality of life. He cites published studies indicating that 80–90% of pregnant mothers who are told they will give birth to a child with Down's syndrome are likely to terminate the pregnancy. Yet, "they have little idea of what it means to have a child with Down's syndrome or to be a person with Down's syndrome," says Reeves. He notes that people with Down's syndrome have become actors, authors and musicians — feats many of us only aspire to. And just in the past two years, he says, several studies have made breakthroughs in developing pharmacological approaches to address cognitive deficits that will allow those with Down's syndrome to live even fuller lives.

Reeves sees a great irony in the fact that although their quality of life is often disavowed, it is the genomes of those with three copies of chromosome 21 that may ultimately yield a key to cancer prevention. "If trisomy 21 weren't compatible with a full life, it is unlikely that a study such as this would have been undertaken, let alone funded," he says. "Who would be foolish enough to randomly overexpress genes thought to cause cancer in order to prevent it?" ■

FROM THE BLOGOSPHERE

For those concerned about the effects of conference air travel on the environment, Second Nature, NPG's archipelago in Second Life (www.secondlife.com), was the virtual venue for a series of talks coinciding with the United Nations climate-change conference held in Bali in December (see Joanna Scott's blog for details: http://network.nature.com/blogs/user/joannascott).

Tara LaForce from Imperial College London spoke about whether and how we might capture carbon dioxide from power plants, compress it, and store it long-term in various geological structures such as oil reservoirs and deep saline aquifers. And, in another lecture, Euan Nisbet of Royal Holloway University in Surrey, UK, talked about the necessity for accurate monitoring of the climate, greenhouse gases and 'top producers' to have any realistic hope of tackling global warming. Both of these talks, and their associated slides, are available through Scott's blog.

If you are interested in giving your own research talk in this global environment-friendly format, please contact Joanna via her blog, or find her in Second Life, where she is known as Joanna Wombat. ■

Visit Nautilus for regular news relevant to *Nature* authors ▸ http://blogs.nature.com/nautilus and see Peer-to-Peer for news for peer-reviewers and about peer review ▸ http://blogs.nature.com/peer-to-peer.

nature

www.nature.com/nature

Vol 451 Issue no. 7174 | 3 January 2008

EDITORIAL

LONDON nature@nature.com
The Macmillan Building, 4 Crinan Street, London N1 9XW
Tel: +44 (0)20 7833 4000 Fax: +44 (0)20 7843 4596/7
EDITOR-IN-CHIEF: Philip Campbell
PUBLISHING EXECUTIVE EDITOR: Maxine Clarke
EDITORIALS: Philip Campbell, Colin Macilwain
NEWS/FEATURES/ONLINE NEWS: Oliver Morton, Geoff Brumfiel, Daniel Cressey, Michael Hopkin, Nicola Jones, Anna Petherick, Katharine Sanderson, Sarah Tomlin, Gaia Vince
BUSINESS NEWS: Colin Macilwain
BOOKS & ARTS/CORRESPONDENCE & ESSAYS/COMMENTARIES: Sara Abdulla, Joanne Baker, Lucy Odling-Smee, Sarah Tomlin
NEWS AND VIEWS: Tim Lincoln, Andrew Mitchinson, Sadaf Shadan, Richard Webb
PHYSICAL, CHEMICAL AND EARTH SCIENCES: Karl Ziemelis, Rosamund Daw, Joshua Finkelstein, Magdalena Helmer, Juliane Mössinger, Karen Southwell, Joanna Thorpe, John VanDecar, Liesbeth Venema
BIOLOGICAL SCIENCES: Ritu Dhand, Lesley Anson, Tanguy Chouard, Henry Gee, Marie-Thérèse Heemels, Rory Howlett, Claudia Lupp, Barbara Marte, Deepa Nath, Ursula Weiss **INSIGHTS/REVIEWS/PROGRESS:** Lesley Anson
SUBEDITORS: Colin Sullivan, Sarah Archibald, Catherine Cassidy, Davina Dadley-Moore, Isobel Flanagan, Paul Fletcher, Jenny Gillion, Dinah Loon, David Price, Chris Simms, Anna York
EDITORIAL PRODUCTION: James McQuat, Alison Hopkins, Marta Rusin, Charles Wenz, Lauren Wethmar **MANUFACTURING PRODUCTION** Jenny Henderson, Stewart Fraser Susan Gray, Jocelyn Hilton, Yvonne Strong
ART AND DESIGN: Martin Harrison, Wesley Fernandes, Madeline Hutchinson, Barbara Izdebska, Paul Jackman, Fern McNulty, Nik Spencer
ADMINISTRATION: Pauline Haslam, Karen Jones, Helen Anthony, Lisa Griffin, Jayne Henderson, Aimee Knight, Alison McGill, Jenny Meyer, Alison Muskett, Nichola O'Brien
PRESS OFFICE: Ruth Francis, Katherine Anderson, Rachel Twinn

WASHINGTON DC nature@naturedc.com
968 National Press Building, 529 14th St NW, Washington DC 20045-1938
Tel +1 202 737 2355 Fax. +1 202 628 1609
EDITORIAL: Eric Hand, Gene Russo, Leslie Sage, Jeff Tollefson, Alexandra Witze
ADMINISTRATION: Katie McGoldrick, Kenneth Simpson

NEW YORK nature@natureny.com
75 Varick St, 9th Floor, New York, NY 10013-1917
Tel +1 212 726 9200 Fax: +1 212 696 9006
EXECUTIVE EDITOR: Linda Miller
EDITORIAL: I-han Chou, Chris Gunter, Kalyani Narasimhan, Helen Pearson

BOSTON nature@boston.nature.com
25 First Street, Suite 104, Cambridge, MA 02141
Tel: +1 617 475 9275 Fax: +1 617 494 4960
EDITORIAL: Angela Eggleston, Joshua Finkelstein, Heidi Ledford **ADMINISTRATION:** Eric Schwartz

SAN FRANCISCO nature@naturesf.com
225 Bush Street, Suite 1453, San Francisco, CA 94104
Tel +1 415 403 9027 Fax: +1 415 781 3805
EDITORIAL: Erika Check Hayden, Natalie DeWitt, Alex Eccleston
ADMINISTRATION: Jessica Kolman

SAN DIEGO r.dalton@naturesf.com
3525 Del Mar Heights Road, PMB No. 462, San Diego, CA 92130
Tel. +1 858 755 6670 Fax. +1 858 755 8779
EDITORIAL: Rex Dalton

MUNICH a.abbott@nature.com
Josephspitalstrasse 15, D-80331 München
Tel +49 89 549057-13 Fax. +49 89 549057-20
EDITORIAL: Alison Abbott, Quinn Schiermeier

PARIS d.butler@nature.com
2 rue Moreau Vincent, 37270 Véretz Tel. +33 2 47 35 72 15
EDITORIAL: Declan Butler

TOKYO editnature@natureasia.com
Chiyoda Building 5-6th Floor, 2-37 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162-0843
Tel +81 3 3267 8751 Fax +813 3267 8754
EDITORIAL: David Cyranoski, Mika Nakano, Akemi Tanaka

CONTRIBUTING CORRESPONDENTS
AUSTRALASIA: Carina Dennis Tel: +612 9404 8255
INDIA: K. S. Jayaraman Tel: +9180 2696 6579
ISRAEL: Haim Watzman Tel +972 2 671 4077
SOUTH AFRICA: Michael Cherry Tel: +27 21 886 4194
WASHINGTON DC: Meredith Wadman Tel +1 202 626 2514
MISSOURI: Emma Marris Tel: +1 573 256 0611

NATURE ONLINE

www.nature.com/nature

CHIEF TECHNOLOGY OFFICER: Howard Ratner **PUBLISHING DIRECTOR, NATURE.COM:** Timo Hannay
WEB PRODUCTION/DESIGN: Jeremy Macdonald, Glennis McGregor, Alexander Thurrell
WEB PRODUCTION TECHNOLOGIES: Heather Rankin **APPLICATION DEVELOPMENT:** Peter Hausel
NATURE PODCAST: Adam Rutherford, Kerri Smith, Sara Abdulla

PUBLISHING

LONDON feedback@nature.com
The Macmillan Building, 4 Crinan Street, London N1 9XW
Tel: +44 (0)20 7833 4000 Fax: +44 (0)20 7843 4596/7
MANAGING DIRECTOR: Steven Inchcoombe
PUBLISHER: Sarah Greaves
ASSISTANT PUBLISHER: Samia Mantoura **PUBLISHING ASSISTANT:** Claudia Banks

TOKYO feedback@natureasia.com
Chiyoda Building 5-6th Floor, 2-37 Ichigaya Tamachi, Shinjuku-ku, Tokyo, 162-0843
Tel: +81 3 3267 8751 Fax: +81 3 3267 8754
PUBLISHING DIRECTOR — ASIA-PACIFIC: David Swinbanks
ASSOCIATE DIRECTOR — ASIA-PACIFIC: Antoine E Bocquet

DISPLAY ADVERTISING

MANAGEMENT: John Michael

NORTH AMERICA display@natureny.com
NEW ENGLAND: Sheila Reardon
Tel: +1 617 494 4900 Fax +1 617 494 4960
NEW YORK/MID-ATLANTIC/SOUTHEAST: Jim Breault
Tel: +1 212 726 9334 Fax. +1 212 696 9481
MIDWEST: Mike Rossi
Tel: +1 212 726 9255 Fax +1 212 696 9481
WEST: George Lui
Tel: +1 415 781 3804 Fax +1 415 781 3805

EUROPE/REST OF WORLD display@nature.com
GERMANY/SWITZERLAND/AUSTRIA: Sabine Hugi-Fürst
Tel: +41 52 761 3386 Fax: +41 52 761 3419
UK/IRELAND/DENMARK/FRANCE/BELGIUM/EASTERN EUROPE: Jeremy Betts
Tel: +44 (0)20 7843 4959 Fax, +44 (0)20 7843 4749
SCANDINAVIA (EXCL. DK)/THE NETHERLANDS/ITALY/SPAIN/PORTUGAL/ISRAEL/ICELAND:
Graham Combe Tel +44 (0)20 7843 4969 Fax: +44 (0)20 7843 4749

JAPAN display@natureasia.com
Kate Yoneyama, Ken Mikami
Tel: +813 3267 8765 Fax +813 3267 8746

SPONSORSHIP

EUROPE/NORTH AMERICA e.green@nature.com
NATURE BUSINESS DEVELOPMENT EXECUTIVE: Emma Green
Tel: +44 (0)20 7833 4000 Fax: +44 (0)20 7843 4749

NATUREJOBS

naturejobs@nature.com

Please refer to panel at the start of the *NatureJobs* section at the back of the issue

MARKETING & SUBSCRIPTIONS

USA/CANADA/LATIN AMERICA subscriptions@natureny.com
Nature Publishing Group, 75 Varick St, 9th Floor, New York, NY 10013-1917
Tel: (USA/Canada) +1 866 363 7860; (outside USA/Canada) +1 212 726 9365
MARKETING: Sara Girard **FULFILMENT:** Karen Marshall

JAPAN/CHINA/KOREA subscriptions@natureasia.com
Chiyoda Building 5-6th Floor, 2-37 Ichigaya Tamachi, Shinjuku-ku, Tokyo, 162-0843
Tel: +81 3 3267 8751 Fax: +81 3 3267 8746
MARKETING/PRODUCTION: Keiko Ikeda, Takesh Murakami

EUROPE/REST OF WORLD subscriptions@nature.com
Nature Publishing Group, Subscriptions, Brunel Road, Basingstoke, Hants RG21 6XS, UK
Tel: +44 (0)1256 329242 Fax +44 (0)1256 812358
MARKETING: Katy Dunningham, Elena Woodstock

INDIA npgindia@nature.com
Nature Publishing Group, 3A, 4th Floor, DLF Corporate Park, Gurgaon 122002
Tel: +91 124 2881053/54 Fax: +91 124 2881052
HEAD OF BUSINESS DEVELOPMENT, INDIA: Jaishree Srinivasan **MARKETING:** Harpal Singh Gill

Annual subscriptions (including post and packing)

INSTITUTIONAL/CORPORATE RATE:	$2,730
PERSONAL RATE:	$199
STUDENT RATE:	$99
POSTDOC RATE:	$119

Printed in USA. Individual rates available only to subscribers paying by personal check or credit card. Orders for student/postdoc subscriptions must be accompanied by a copy of student ID. Rates apply to USA, Canada, Mexico/Central & South America.
Add 7% GST tax in Canada (Canadian GST number 140911595).
BACK ISSUES: US$20.00

SITE LICENSES FULFILMENT & CUSTOMER SERVICES

feedback@nature.com

SITE LICENSES: npg.nature.com/libraries
FULFILMENT: Dominic Pettit
CUSTOMER SERVICE: Gerald Coppin

ELECTROCOMPETENT STRAINS NOW AVAILABLE

NEW ENGLAND BIOLABS

Competent Cells from New England Biolabs

COMPLETING YOUR CLONING NEEDS

Make the switch to competent cells from New England Biolabs to help bring success to your research. Our expanded line of competent cells includes a variety of strains for cloning and expression, as well as strains with unique properties (see chart). For added convenience we offer a choice of efficiencies, formats and customized packaging. Now you can digest, ligate and transform with reagents from the name you trust.

Advantages

- Extremely high transformation efficiencies
- Phage T1 resistance (*fhuA2*) preserves clone integrity
- Choice of protocols: high efficiency or 5 minute transformation
- Nonspecific endonuclease activity eliminated, resulting in highest quality plasmid preparations
- Express difficult or toxic proteins with T7 Express strains containing $lacI^q$ and/or a novel lysY variant
- Obtain colonies faster than any other commercial strain with NEB Turbo
- SOC Outgrowth Media and pUC19 Control Plasmid included
- Free of animal products

Cloning strain characteristics	Strain	NEB #
Obtain colonies faster than any other commercial strain (6.5 hours)	NEB Turbo Competent *E. coli*	C2984H/I
Versatile cloning strain		C2987H/I
Cloning of [illegible] genes	NEB 5-alpha F′ I^q Competent *E. coli*	[illegible]
Cloning of large plasmids and BACs		C3019H/I
Growth of unmethylated plasmids	[illegible] Competent *E. coli*	C2925H/I

Expression strain characteristics	Strain	NEB #
Most popular non-T7 protein expression strain	NEB Express Competent *E. coli*	C2523H/I
Added control of IPTG induced expression with non-T7 plasmids		C3037H/I
Most popular T7 protein expression strain		C2566H/I
[illegible] expression	T7 Express I^q Competent *E. coli*	[illegible]
Tight control of protein expression by inhibition of T7 RNA Polymerase		C3010H/I
Highest level of protein expression control		C3013H/I
For crystallography experiments/SeMet labeling		C3022H/I

† *Available as subcloning efficiency* * *Available as electrocompetent cells*

For more information and our international distribution network, please visit www.neb.com
For a copy of our new Competent Cell Brochure, please visit www.neb.com/literaturerequest

New England Biolabs Inc. 240 County Road, Ipswich, MA 01938 USA 1-800-NEB-LABS Tel. (978) 927-5054 Fax (978) 921-1350 info@neb.com
Canada Tel. (800) 387-1095 info@ca.neb.com • **China** Tel. 010-82378266 beijing@neb-china.com • **Germany** Tel. 0800/246 5227 info@de.neb.com
Japan Tel. +81 (0)3 5669 6191 info@neb-japan.com • **UK** Tel. (0800) 318486 info@uk.neb.com

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

nature

www.nature.com/nature

Vol 451 | Issue no. 7174 | 3 January 2008

Election fireworks

Now is the time for the research community to catch the attention of the next president of the United States of America.

Every New Year is an occasion for celebration, tinged with uncertainty. This year begins with financial markets in a particularly uncertain state, with no banker or citizen quite certain what fresh crisis lurks around the corner.

One thing we can be sure of is that all of us — from Maryland to Mozambique — will be hearing plenty during 2008 about an unusually open election for the next president of the United States. By this time next year, a new person will be set to assume that role, with major ramifications for scientists, as for everybody else.

Two major science-related issues — the rules for the conduct of embryonic stem-cell research, and society's response to climate change — are likely to feature fairly prominently in the run-up to the 4 November election. A host of secondary science and technology issues, such as agency budgets (see page 2) and whether the United States embarks on ambitious technology-policy initiatives in a bid to bolster its industrial competitiveness, will hinge on the election's outcome.

The process by which the two main parties select their candidates kicks off at the Iowa caucuses today. One interesting aspect is that several candidates offer approaches on these major issues that are not normally associated with their respective parties (see page 4).

This diversity is particularly striking among the Republicans, where the race remains extraordinarily wide open. For example, Senator John McCain (Republican, Arizona), unlike most in his party, has long championed decisive action to confront climate change. Two of his leading rivals, Mitt Romney and Rudy Giuliani, are supportive of federal funding for human embryonic stem-cell research. The leading candidates on the Democrat side are less diverse in their views. Whether that signifies a party that is healthily united or unhealthily moribund is for the voters to decide.

Election year offers a chance for scientists who aspire to a direct role in the political process to make their voices heard. Prompted by seven years of what they see as manipulation of scientific findings by the Bush administration, groups are trying to raise the profile of science in the upcoming campaign. An organization called Scientists and Engineers for America plans to launch a project tracking the science- and health-related votes of all members of Congress, plus challengers for their seats as well as the presidential candidates. Meanwhile, dozens of prominent scientific leaders have mounted a push for a 'Science Debate 2008', calling for a candidates' debate on science and technology issues.

It's a laudable idea, and even if the prospects of such a debate are rather remote, the campaign can play a useful role in raising the profile of important issues as the election unfolds. For it is now — while candidates are striving to win their respective party nominations — that their priorities, preferences and policy teams will be forged. Many researchers, of all political stripes, are deeply troubled by what they regard as the dysfunctional relationship between science and the outgoing Bush administration. There is a better chance of a more fruitful relationship arising next time round if scientists get involved early with the candidates, and with the energetic, nationwide public debate that already characterizes this most intense and open of primary seasons. ■

Think about it

Reliable policy research is an underrated planning tool in developing countries.

Step out onto any of Jakarta's city-centre highways during office hours and you will have no trouble crossing the road. This is because during peak times, a car journey that should take 20 minutes can take up to two hours, as drivers crawl along in the 30 °C heat.

If Jakarta was the capital of a developed country, the authorities would have access to science-based advice on the policy options for easing the traffic from universities, state-sponsored research centres, industry, environmental groups and think-tanks. But in much of Africa, Asia, Latin America and the Middle East, such advice is rarely available for areas such as transport, agriculture, health, education and energy.

The lack of this capacity in Indonesia has recently been highlighted by the World Bank, which is considering a proposal to create a policy think-tank geared specifically to the needs of the many international agencies that are operating in the country.

The bank's entry into this sphere is welcome. A couple of decades ago, such agencies seemed uninterested in helping young researchers from developing countries to gain badly needed skills in policy analysis. As a result, the African Centre for Technology Studies in Nairobi, for example, began life in the spare bedroom of its founder, Calestous Juma. And Saleemul Huq, founder of the Bangladesh Centre for Advanced Studies, spent many a sleepless night worrying about how to pay his staff.

Today, these two centres are among the developing world's leading research-policy establishments, and their histories, along with those of Pakistan's Sustainable Development Policy Institute and Colombia's Alexander von Humboldt Institute, are described on page 8.

Two lessons stand out from these stories. The first is that each institution relied heavily on a driven, committed individual, who nurtured success where many would have expected failure. The second is that, despite the success of these particular institutions, impartial policy analysis is being held back in poor countries for want of either public or private sector support within these nations themselves.

Both governments and wealthy individuals in developing countries continue, on the whole, to regard sound policy analysis as a luxury that they cannot afford. They are wrong, and should join with donor nations and international agencies in backing the establishment of reliable, local organizations that can undertake it. ■

NEWS


P GINTER

Taking a hit: high-energy physics at Fermilab faces severe cuts in funds in the budget approved by Congress.

Budget blow to US science

It was an imperative that was supposed to transcend party politics. The America COMPETES Act, put forth by congressional Republicans and Democrats and signed into law by President Bush in August, was meant to signal support for boosting basic science in the name of remaining competitive internationally

But in a mammoth $555-billion spending bill passed by Congress on 19 December, funding for basic science took a beating. Gone are plans to double funding at the National Science Foundation (NSF) and the Office of Science of the Department of Energy (DOE). "It's dead in this budget," says Samuel Rankin, Washington DC office director for the American Mathematical Society and chair of the Coalition for National Science Funding. "Hopefully we can resurrect that feeling again next year"

The spending bill marks the end of the annual budget wrangling in Congress (see *Nature* **449**, 962; 2007). It includes spending for all government departments other than defence, which has already been approved. The final numbers for fiscal year 2008 (see table) include what amounts to a 0.5% increase for the National Institutes of Health (NIH), less than one-sixth of the rise that Congress had sought in an earlier, unsuccessful bill. Within the physical sciences, programmes in high energy physics and fusion are hit particularly hard. "This is probably the worst budget for science that anyone can remember," says Michael Lubell, a spokesman at the American Physical Society in Washington DC. "It absolutely devastates and probably wipes out American high-energy physics."

TOTAL AGENCY BUDGETS 2008 (US$ million)

	FY 2007	FY 2008
NIH	28,809	28,941
NASA	16,267	17,117
NSF	5,916	6,032
NOAA	4,078	3,896
DOE Office of Science	3,797	3,973

Democrats blame veto threats from the Bush administration, which forced a last-minute showdown over the size of spending on domestic programmes such as science. The Republican version of the story is that Congress could have kept the domestic priorities if it hadn't been for thousands of special 'earmarked' projects worth billions of additional dollars.

> "It probably wipes out American high-energy physics."

One Democratic priority, energy research, fared relatively well; Congress ignored a flat presidential request and instead boosted research and development (R&D) money for renewables, energy efficiency and nuclear energy by 30%, to nearly $1.3 billion, according to an analysis by budget expert Kei Koizumi at the American Association for the Advancement of Science. The bill also boosts funding for clean coal and other fossil fuel R&D activities by 13%, to $557 million. Overall funding for nuclear-energy programmes increased, although the president's request for the Global Nuclear Energy Partnership, to hasten work on reprocessing nuclear waste, was cut by more than half, to $181 million.

The DOE Office of Science took bigger hits. Although its total science budget grew 4.6% to $4 billion, most of those increases were for supercomputers and biological research. Congress withheld money for the energy department's $160-million commitment to ITER, the international fusion reactor in France, and slashed funding for the International Linear Collider (ILC), the next-generation particle accelerator, from $60 million to $15 million.

Most of the ILC money would have gone to Fermilab in Batavia, Illinois. The lab also saw its NOvA neutrino experiment programme eliminated. Fermilab's director, Pier Oddone, told his staff to expect 200 lay-offs in the spring (from a 1,900-person workforce), along with mandatory unpaid leave for remaining employees. The lab may even temporarily shut down its Tevatron accelerator.

Elsewhere within the energy department, at the National Nuclear Security Administration, money was found for non-proliferation and verification, which saw a 43% increase to $387 million, at the expense of designing and maintaining nuclear weapons. The Reliable Replacement Warhead programme, to develop a new generation of nuclear weapons (see *Nature* **442**, 18–21; 2006), was cut completely.

The National Science Foundation saw its R&D funding grow 1%, to $4.5 billion — not the 8% rise requested by Bush.

At the National Oceanic and Atmospheric Administration, R&D funding rose 7.6%, to $573 million. At the National Institute of Standards and Technology, total R&D funding rose nearly 5% to $514 million, but within that the agency's main research programme drops 0.8% from $372 million in the previous year.

NASA saw a 5.7% increase in its overall R&D budget, to $12.5 billion. But much of that money is tied to completion of the International Space Station and for rockets to return astronauts to the Moon. Within the $5.5-billion science directorate, Earth sciences received the biggest boost — of 4.4%, to $1.5 billion — while planetary science suffered the most, with a 1.4% cut, to $1.4 billion.

The NIH, which had been set to receive a 3.1% boost in a budget bill vetoed by Bush in November (see *Nature* **450**, 470, 2007), will instead get a 0.5% increase of $133 million, bringing its effective budget to $28.9 billion. That will make 2008 the fifth consecutive year of effectively flat funding for the NIH.

The 0.5% increase drew sharp rebukes from advocates for biomedical research, who criticized Bush for forcing Congress to shave more than half a-billion dollars from what it had allotted to the NIH in November. "That was a really big hit," says David Moore, senior associate vice-president at the Association of American Medical Colleges in Washington DC. "We're extremely disappointed," adds Jon Retzlaff, the senior lobbyist at the Federation of American Societies for Experimental Biology.

Still, some corners of the NIH will have reason to celebrate, such as the once embattled National Children's Study, which Bush had tried to eliminate but which ended up growing by $42 million, to $111 million. Open-access advocates also applauded a provision in the bill that will require NIH-funded investigators to submit — or have submitted for them — their peer-reviewed manuscripts to the National Library of Medicine's PubMed Central when they are accepted for publication. The manuscripts will be made publicly available no later than 12 months after publication.

Meanwhile, Speaker of the House Nancy Pelosi (Democrat, California) sent out a letter to the research community, saying that "her commitment to the innovation agenda remains strong and steadfast". And advocates of the physical sciences vowed to keep fighting. Charles Vest, president of the National Academy of Engineering, says that other countries are proceeding apace with research investments. "If we keep doing business as usual," he says, "we're going to get our lunch eaten." ■

Eric Hand, with additional reporting by Meredith Wadman and Jeff Tollefson

See Editorial, page 1.

HAVE YOUR SAY
Comment on any of our news stories, online.
www.nature.com/news

Airgun ban halts seismic tests

Geologists hoping to study Earth's crust off the coast of British Columbia have reached an impasse with the Canadian government, delaying their long-planned research projects. Canada has not issued permits for geological work using airguns — which fire bursts of air into the ocean — on the basis that it may disturb marine life, including whales. The dispute is so intense that one long-planned US$2.5-million project is "dead in the water". A second study, meant to facilitate a Can$100-million (US$99-million) Canadian seafloor observatory system, has been delayed at least three months, if not indefinitely.

The researchers are exasperated, arguing that they have done "everything right" to comply with environmental protection laws. They say that Canadian agencies have capitulated to environmental organizations.

Canada has a moratorium on oil and gas development, which also involves airguns to locate reserves, along its western coastal waters. Fears that a scientific airgun cruise could open the waters to oil and gas exploration sunk the research projects' chances, says Lincoln Hollister, a geoscientist at Princeton University in New Jersey. Hollister has spent more than four years trying to win Canadian approval to use airguns to map the crust beneath the mountains and coastal fjords of Queen Charlotte Sound in northern British Columbia.

In 1994, he led a similar cruise without causing any environmental harm, he says. The team has footage of a humpback whale basking undisturbed in the distant background while airguns were fired.

Anecdotes aside, there is no definitive data yet available on the effects such seismic tests have on marine animals. Results from a US study on this conducted in summer 2007 have yet to be published.

In March, the Natural Sciences and Engineering Research Council of Canada (NSERC) killed plans to provide Can$300,000 for the Canadian members of Hollister's team. NSERC environmental officer Diane Fraser in Ottawa says that this was done on advice from scientists at the Department of Fisheries and Ocean (DFO). "There were not enough scientific data to be able to determine one way or another if airguns would be harmful," says Fraser.

Since that rejection, Hollister says he has attempted repeatedly to learn from the DFO and NSERC what could done to remedy the situation, but no one responds. Adam Silverstein, an environmental-assessment manager at the DFO in Vancouver, denies knowledge of such requests. Hollister counters that Silverstein was repeatedly copied in on e-mail. "It is widely recognized that everything was done right to get the permits," Hollister claims.

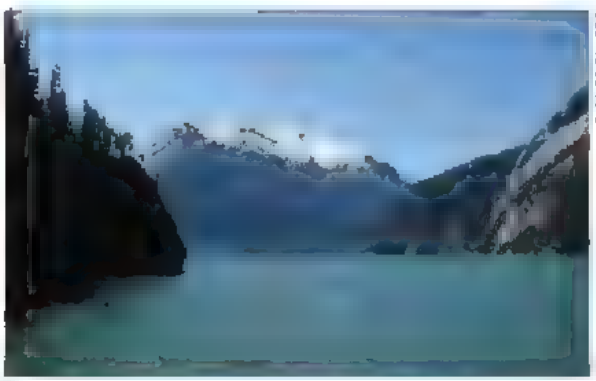
Margot Venton, a legal consultant for environmental group Ecojustice in Vancouver, acknowledges that the potential for oil and gas exploration was a concern, and says that as Canadian agencies failed to show the acoustic study would not cause harm to animals, it shouldn't proceed.

At the US National Science Foundation (NSF), which funds the US component of Hollister's study, there is dismay, notes William Lang, who secures environmental permits for NSF-funded scientists in foreign waters. "The very high-value proposal didn't get a fair hearing in the public forum," he says.

The airgun issue is also thwarting plans for the *Marcus Langseth*, a US$20-million research vessel operated by the Lamont-Doherty Earth Observatory at Columbia University in New York City. The ship was intended to be in Canadian waters next July to measure seismic velocities in preparation for the installation of Neptune Canada — a seafloor observatory — in the summer of 2009, says Douglas Toomey, a geologist at the University of Oregon in Eugene. But the US$2-million cruise has now been delayed until at least October.

A Lamont-Doherty spokesman says that US state department officials are seeking "reasonable assurance" that the Toomey project will secure a permit before an expensive Canadian application process is initiated. ■

Rex Dalton



C. ANDRONICOS

Concerns about whales have blocked use of airguns in Canada.

IOWA AND AFTER

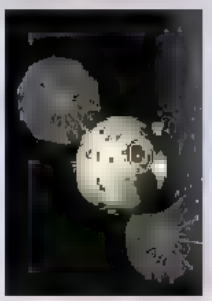
After months of manoeuvre and preliminary debate, on 3 January the Iowa caucuses mark the start of the formal process by which the US political parties will choose their presidential candidates. A host of issues are on the table, and science is not high on the list. Nevertheless some scientific issues are cropping up out on the campaign trail.

Stem cells, although still an issue, seem to have taken a back seat, even though the new president would be in a position to repeal President George W. Bush's 2001 restrictions on federal funding for research on embryonic stem-cell lines. Climate and energy receive a lot of attention, with candidates happy to talk of emissions reductions that would bite far after their terms have ended, and with some who have previously opposed corn-ethanol subsidies changing their minds when looking for votes among the Iowa cornfields. Spending on the physical sciences, other than energy, comes up rarely — although Senator Barack Obama's proposal to take funds from NASA's planned shuttle replacement has ruffled some feathers.

Nature's Eric Hand takes a look at the leading candidates' stances on some science-related issues.

See Editorial, page 1.

CLIMATE/ENERGY	Calls for reducing emissions by 80% from 1990 levels by 2050 via a cap-and-trade system. Does not support a carbon tax but argues for standards on efficiency, mileage, and renewable energy to meet that goal. Says she is "agnostic" about nuclear power.	Calls for reducing emissions by 80% from 1990 levels by 2050 via a cap-and-trade system. Wants to invest $150 billion over 10 years in alternative energy sources. Supports coal liquefaction, but only if it emits 20% less carbon than conventional fuels.	Calls for reducing emissions by 80% from 1990 levels by 2050 via a cap-and-trade system. Wants to push for a climate change treaty that has binding elements for all countries, including those in the developing world. Opposes nuclear power — says it's too costly to build new plants and too unsafe to dispose of waste.	Calls for reducing emissions by 90% from 1990 levels by 2050 via a cap-and-trade system. Proposes increasing mileage standards to 50 miles per gallon by 2020, and setting a renewable-energy target of 50% by 2040.	Calls for reducing emissions by 80% from 1990 levels by 2050. Wants every US car sold to be equipped with flex-fuel technology, and half of all major gas stations to offer biofuels by 2017. Supports ethanol from corn as a transitional solution for energy woes, but says it is not sustainable in the long term and pushes for cellulosic sources.
BIOMEDICAL/STEM CELLS	Supports federal funding for embryonic stem-cell research. Proposed increasing National Institutes of Health budget by 50% over five years, and doubling it over ten years.	Supports federal funding for embryonic stem-cell research.	Supports federal funding for embryonic stem-cell research.	Supports federal funding for embryonic stem-cell research. Proposed a state-funded embryonic stem-cell research centre at the University of New Mexico in Albuquerque.	Supports federal funding for embryonic stem-cell research.
SPACE	Supports human exploration of space, including completing the International Space Station and replacing the space shuttle with a new generation of launch vehicles.	Would delay NASA's Constellation programme to build new rockets and crew vehicles for five years, instead putting that money toward an $18-billion education plan.	Supports human exploration of space, and says other countries should also be involved.	Sees space as a "bona fide area of economic growth and opportunity". Pushed for a sales tax to support the building of a spaceport in New Mexico.	Wants to make China a full partner in space exploration rather than a "frustrated new entrant" that has to catch up with the United States.
NOTABLE QUOTE	"My 5th grade teacher Mrs Kraus came into our classroom and said we had to study math and science because the President said so. I was convinced that President Eisenhower had called up Mrs Kraus and told her, 'You tell those children — and particularly that Hillary, who doesn't really like math — that her country needs her'."	"We're not going to have the engineers and scientists to continue space exploration if we don't have kids who are able to read, write and compute."	"Colleges are the places where we ensure that America is competitive. Yet we've taken away funding for the NIH and our research universities. That's just a mistake."	"I myself have been told that I have a lot of energy. The secret is that I use renewable resources. Some days I'm solar powered. Some days I'm wind powered. And some days my critics just think I'm full of compressed air."	"For too long we have abdicated the responsibility to reduce our own emissions."

DENNIS KUCINICH (DEMOCRAT) REPRESENTATIVE FROM OHIO	RUDY GIULIANI (REPUBLICAN) FORMER MAYOR OF NEW YORK CITY	MITT ROMNEY (REPUBLICAN) FORMER GOVERNOR OF MASSACHUSETTS	MIKE HUCKABEE (REPUBLICAN) FORMER GOVERNOR OF ARKANSAS	JOHN MCCAIN (REPUBLICAN) SENATOR FROM ARIZONA	FRED THOMPSON (REPUBLICAN) TELEVISION ACTOR AND FORMER SENATOR FROM TENNESSEE	RON PAUL (REPUBLICAN) REPRESENTATIVE FROM TEXAS
Calls for reducing emissions by 80% from 1990 levels by 2050, potentially via a carbon tax. Proposes a Works Green Administration that would retrofit buildings with wind and solar power. Wants to halt all mining and logging on public land.	Supports expanding nuclear power and ethanol subsidies. Does not support increasing mileage standards or mandatory caps on emissions.	In 2004 launched a plan to address global warming, though at the time he questioned if it was happening. Pulled out of a New England emission plan (implemented by his successor). Favours tariff protection for the corn-ethanol industry. "We should not exchange dependence on oil from other countries for dependence on sugar cane from Brazil."	Supports expanding nuclear power and raising mileage standards. "It's a sin against future generations for me to act as if there are no future generations that should enjoy the world as I do."	Has been a leader in the Senate on global-warming legislation. Introduced a bill with Joseph Lieberman (Ind, Connecticut) to cut emissions by roughly 60% from 1990 levels by 2050. Does not support a carbon tax. Once criticized ethanol as an alternative fuel, but now supports it, citing the rising cost of oil.	Says there is no scientific consensus about the cause of global warming, but that it makes sense to take "reasonable steps" to reduce emissions without harming the economy. Voted against ethanol subsidies but now says he supports them. Voted against raising mileage standards and for drilling in the Arctic National Wildlife Refuge.	On global warming: says there are "reputable scientists on both sides of that argument". Opposes major government regulations to control emissions. Does not support a carbon tax, which he calls "simply an acknowledgement that you can pollute with government permission". Supports expanding nuclear energy. Opposes tariffs on ethanol from corn.
Supports federal funding for embryonic stem-cell research. "There are so many different things that stem-cell research can teach us."	Supports federal funding for embryonic stem-cell research from surplus embryos from fertilization clinics. "As long as we're not creating life in order to destroy it, as long as we're not having human cloning, and we limit it to that ... there is plenty of opportunity to then use federal funds in those situations where you have limitations."	Calls for a ban on creating embryos for research purposes, but does not oppose the use of surplus embryos from *in vitro* fertilization clinics. Does not support federal funding for the research.	Opposes federal funding of embryonic stem-cell research, and points to work on adult stem cells instead.	Supports embryonic stem-cell research. "I believe that we need to fund this. This is a tough issue for those of us in the pro-life community. I would remind you that these stem cells are either going to be discarded or perpetually frozen. We need to do what we can to relieve human suffering."	Opposes stem-cell research that requires the destruction of human embryos. Says that embryonic research hasn't had a breakthrough, and cites a disputed list of 73 'breakthroughs' that use adult stem cells.	Says individual states and citizens should decide whether to permit, ban or fund embryonic stem-cell research. "I strongly object to forcing those Americans who believe embryonic stem-cell research is immoral to subsidize such research with their tax dollars."
Wants NASA to focus "more on earthly projects" such as producing green energy. Says innovations will "eventually" be turned into space exploration.	Says he will continue to aggressively support space exploration. In response to a child's question, said he would be prepared to defend against an alien attack.	Supports the space programme as a way to drive investment in technology and innovation. Says he has no reason to change Bush's plan for NASA.	Broadly supports space exploration as a trigger for technology development. "Now whether we need to send somebody to Mars, I don't know. But I'll tell you what: If we do, I've got a few suggestions, and maybe Hillary could be on the first rocket to Mars."	Showed some scepticism after Bush's initial announcement, but now calls it "not only visionary, but doable."	Has said little except... 	Says he is "absolutely committed to human space exploration".
On reports that he had seen a UFO: "More people in this country have seen UFOs than I think approve of George Bush's presidency." 	"Nuclear power is dangerous. So is every form of power. But no one's died from nuclear power in the United States. So our commitment here is to expand it, make sure it's safe."	"I believe that God designed the Universe and created the Universe. I believe he used the process of evolution to create the human body."	"If you want to believe that you and your family came from apes, I'll accept that."	On the push for global-warming legislation: "Inaction could be disastrous."	... "[Reports of rising temperatures on other planets have] led some people, not necessarily scientists, to wonder if Mars and Jupiter, non-signatories to the Kyoto Treaty, are actually inhabited by alien SUV-driving industrialists who run their air conditioning at 60 degrees and refuse to recycle."	"Neither party in Washington can fathom that millions and millions of Americans simply don't want their tax dollars spent on government research of any kind."

Information gathered from questionnaires sent to candidates' campaigns, from their published platforms and from media reports. Images from: K. Lamarque/Reuters; S. Wenig/AP; C. East/Reuters; A. Wong/Getty; E. Miller/Getty; K. Bleier/AFP/Getty; R. Matay/AP; J. Rinaldi/Reuters; Y. Paskova/Getty; E. Dunand/AFP/Getty; B. Pugliano/Getty; M. Carlson/AP; J. Sohm/Visions of America/Corbis; M. Stojkovic/SPL, Royalty-Free/Corbis; Lockheed Martin Corp.

Free journal-ranking tool enters citation market

A new Internet database lets users generate on-the-fly citation statistics of published research papers for free. The tool also calculates papers' impact factors using a new algorithm similar to PageRank, the algorithm Google uses to rank web pages. The open-access database is collaborating with Elsevier, the giant Amsterdam-based science publisher, and its underlying data come from Scopus, a subscription abstracts database created by Elsevier in 2004.

The SCImago Journal & Country Rank database was launched in December by SCImago, a data-mining and visualization group at the universities of Granada, Extremadura, Carlos III and Alcalá de Henares, all in Spain. It ranks journals and countries using such citation metrics as the popular, if controversial, Hirsch Index. It also includes a new metric the SCImago Journal Rank (SJR).

The familiar impact factor created by industry leader Thomson Scientific, based in Philadelphia, Pennsylvania, is calculated as the average number of citations by the papers that each journal contains. The SJR also analyses the citation links between journals in a series of iterative cycles, in the same way as the Google PageRank algorithm. This means not all citations are considered equal; those coming from journals with higher SJRs are given more weight. The main difference between SJR and Google's PageRank is that SJR uses a citation window of three years.

It will take time to assess the SJR properly, experts say. It is difficult to compare the results of SJR journal analyses directly with those based on impact factors, because the databases each is based on are different. Thomson's Web of Science abstracts database covers around 9,000 journals and Scopus more than 15,000, and in the years covered by the SCImago database — 1996 to 2007 — Scopus contains 20–45% more records, says Félix de Moya Anegón, a researcher at the SCImago group.

The top journals in SJR rankings by discipline are often broadly similar to those generated by impact factors, but there are also large differences in position (see table). *Immunity* (SJR of 9.34) scores higher than *The Lancet* (1.65), for example, but *The Lancet*'s 2006 impact factor of 25.8 is higher than the 18.31 of *Immunity*. Such differences can be understood in terms of popularity versus prestige, says de Moya Anegón — popular journals cited frequently by journals of low prestige have high impact factors and low SJRs, whereas journals that are prestigious may be cited less but by more prestigious journals, giving them high SJRs but lower impact factors.

Thomson under fire

The new rankings are welcomed by Carl Bergstrom of the University of Washington in Seattle, who works on a similar citation index, the Eigenfactor, using Thomson data. "It's yet one more confirmation of the importance and timeliness of a new generation of journal ranking systems to take us beyond the impact factor," says Bergstrom, "and another vote in favour of the basic idea of ranking journals using the sorts of Eigenvector centrality methods that Google's PageRank uses."

Thomson has enjoyed a monopoly on citation numbers for years — its subscription products include the Web of Science, the Journal Citation Report and Essential Science Indicators. "Given the dominance of Thomson in this field it is very welcome to have journal indicators based on an alternative source, Scopus," says Anne-Wil Harzing of the University of Melbourne in Australia, who is developing citation metrics based on Google Scholar.

Jim Pringle, vice-president for development at Thomson, says metrics similar to PageRank, such as SJR and Eigenfactor, have proven their utility on the web, but their use for evaluating science is less well understood. "Both employ complex algorithms to create relative measures and may seem opaque to the user and difficult to interpret," he says.

Thomson is also under fire from researchers who want greater transparency over how citation metrics are calculated and the data sets used. In a hard-hitting editorial published in *Journal of Cell Biology* in December, Mike Rossner, head of Rockefeller University Press, and colleagues say their analyses of databases supplied by Thomson yielded different values for metrics from those published by the company (M. Rossner *et al. J. Cell Biol.* **179**, 1091–1092; 2007).

Moreover, Thomson, they claim, was unable to supply data to support its published impact factors. "Just as scientists would not accept the findings in a scientific paper without seeing the primary data," states the editorial, "so should they not rely on Thomson Scientific's impact factor, which is based on hidden data."

Citation metrics produced by both academics and companies are often challenged, says Pringle. The editorial, he claims, "misunderstands much, and misstates several matters", including the authors' exchanges with Thomson on the affair. On 1 January, the company launched a web forum to formally respond to the editorial (see http://scientific.thomson.com/citationimpactforum). ■

Declan Butler

Thomson Scientific Impact Factor (IF) TOP TEN

Rank	Journal	IF	SJR
1	*Ca-A Cancer Journal for Clinicians*	63.3	7.3
2	*New England Journal of Medicine*	51.3	3.7
3	*Annual Review of Immunology*	47.2	22.4
4	*Annual Review of Biochemistry*	36.5	16.1
5	*Reviews of Modern Physics*	33.5	2.7
6	*Nature Reviews Cancer*	31.6	9.2
7	*Physiological Reviews*	31.4	7.9
8	*Nature Reviews Molecular Cell Biology*	31.4	12.2
9	*Science*	30.0	5.3
10	*Cell*	29.2	15.2

SCImago Journal Rank (SJR) TOP TEN

Rank	Journal	SJR	IF
1	*Annual Review of Immunology*	22.4	47.2
2	*Annual Review of Biochemistry*	16.1	36.5
3	*Cell*	15.2	29.2
4	*Annual Review of Cell and Developmental Biology*	14.2	26.6
5	*Nature Immunology*	12.5	27.6
6	*Nature Reviews Molecular Cell Biology*	12.2	31.4
7	*Nature Reviews Immunology*	11.1	28.7
8	*Immunity*	9.3	18.3
9	*Nature Reviews Cancer*	9.2	31.6
10	*Nature Genetics*	9.1	24.2

Debate heats up over food from cloned animals

The stakes were raised last month in the battle to determine whether food from cloned animals and their progeny should be allowed on the shelves of US grocery stores.

In December, Congress urged the Food and Drug Administration (FDA) to keep in place a request that companies voluntarily refrain from selling such foods. It also said that the US Department of Agriculture should study the economic implications of allowing meat and milk from cloned animals into the food supply.

The FDA had been expected to finalize in 2008 a preliminary assessment it made just over a year ago, which concluded that foods from cloned animals and their progeny are not different from products from conventional animals and could probably be sold without special labeling.

On 19 December, the two leading US animal-cloning companies (ViaGen in Austin, Texas, and TransOva Genetics in Sioux Center, Iowa), announced a radio-tracking programme that will use ear tags to follow cloned animals from birth to death. The firms said that the voluntary programme will come into effect as soon as the FDA allows food from clones on the market.

Sociologist to head Turkey's university board

Turkey's president, Abdullah Gül, late last month appointed a little-known sociologist as president of the country's powerful higher-education board, YÖK.

Yusuf Ziya Özcan is considered a moderate in a country where tensions between secularists and Islamists, particularly at universities, have been increasing. His research has included studies on the role of Islam in society. But his surprise appointment — he has little experience of university administration — has not pleased everyone. YÖK's deputy head, engineer Aybar Ertepinar, immediately resigned.

Özcan, from the Middle East Technical University in Ankara, says he wants to lift 'all bans' in universities, implicitly referring to the highly politicized ban on headscarves. He adds that he wants all universities to become autonomous institutions and to "attach a greater importance to being scientific".

The much-debated headscarf ban at Turkish universities may soon be overturned.

REUTERS

California petition to limit vehicle emissions rejected

Arguing that global warming merits a national solution and not "a confusing patchwork of state rules", the US Environmental Protection Agency (EPA) last month rejected a request by California to impose state limits on greenhouse-gas emissions from motor vehicles.

The California regulations would have imposed a reduction of 30% in greenhouse-gas emissions in vehicle exhausts by 2016. Seventeen other states were expected to adopt the standards, which would then have covered around 45% of annual car sales, advocates of the proposal say.

The EPA decision followed federal legislation on 19 December requiring motor-vehicle manufacturers to increase fuel efficiency by 40%, to 35 miles per gallon, by 2020.

Calling the EPA decision "completely absurd", California attorney-general Edmund Brown promised to challenge it in the courts.

Disgraced cloner seeks licence in comeback bid

Korean researcher Woo Suk Hwang, whose 2006 work on human embryonic stem cells turned out to be fabricated, is trying to make a comeback.

Hwang has applied for a new licence to work with human embryonic stem cells, to replace his revoked one. The Korean science ministry is expected to make a decision on the application by April.

His name has also appeared on at least three papers resulting from work done after he was fired from Seoul National University. The privately funded group works at the SooAm Biotech Research Foundation on the outskirts of Seoul. The articles all discuss improvements in pig cloning from new methods of cultivating porcine eggs (Y. W. Jeong *et al. Anim. Reprod. Sci.* doi:10.1016/j.anireprosci.2007.03.021, 2007; E. Lee *et al. Reproduction* 134, 405–414, 2007 and S. L. McElroy *et al. Theriogenology* doi:10.1016/j.theriogenology.2007.10.010, 2007).

Hwang is still on trial on charges of fraud, embezzlement and violation of Korean bioethics laws.

Physicist takes the helm of Italy's research council

Theoretical physicist Luciano Maiani has been nominated president of Italy's national research council, the CNR.

Maiani headed Italy's national institute for nuclear physics from 1993 before taking over at CERN, Europe's particle-physics lab near Geneva, in 1997. But the CNR, which runs 100 or so institutes across the country, may be the toughest challenge so far.

The council has been subject to a stream of ineffectual reforms for the past decade or more, demoralizing the staff and making long-term planning difficult. Maiani, who will take up office in spring, says that his priorities will be to restore the confidence of the scientific staff and get the production of science rolling again.

Maiani was selected by an independent search committee — a new procedure introduced last year by research minister Fabio Mussi to break with the notorious political spoils system.

Illinois picked as site for carbon-capture plant

US DOE/AP

Mattoon, Illinois, is poised to host the first commercial-scale coal-gasification power plant to capture carbon dioxide and pump it underground rather than into the atmosphere. The site sits above a sandstone formation suitable for sequestering carbon dioxide.

The project, called FutureGen (pictured), is supposed to be a showcase for the US Department of Energy, which has promoted technological innovation as a solution to global warming. The department will cover three-quarters of the project's cost, now estimated at $1.8 billion. The rest will come from an alliance of more than a dozen energy companies based in the United States and abroad. Mattoon beat competition from another site in Illinois, and two in Texas. Final approval must still come from the energy department.

NATURE|Vol 451|3 January 2008

ILLUSTRATIONS BY JOSHUA GORCHOV

The road from Rio

Are think-tanks staffed by scientists a luxury that only rich nations can afford? **Ehsan Masood** meets the founders of four institutes that set out to help poorer nations to think for themselves.

It began with Rio. At the 1992 United Nations Earth Summit in Rio de Janeiro in Brazil, 171 countries came together to hammer out global rules to slow down climate change and halt the loss of biodiversity. Then, as now, international meetings on environmental issues see developed countries pitched against developing ones. At such meetings it is all too easy for jaded commentators, and even participants, to conclude that little of consequence would result. Yet they can inspire individuals to take actions that have a lasting effect.

"Rio was a real turning point," says Saleemul Huq, plant biologist and founder of the Bangladesh Centre for Advanced Studies in Dhaka, and now head of the climate change group at the International Institute for Environment and Development in London. "The Rio meeting acted as a wake-up call to developing countries that we needed to raise our game."

During the heady days leading up to Rio, scientists and government officials from developing countries witnessed first hand the power of scientific research in helping to inform and change policies. They saw, for example, how the talks on climate change needed a consensus, not just from politicians, but also from scientists. And they discovered that delegates from richer countries had access to environmental expertise from government, industry and academia.

Huq recalls that before Rio, environmental problems were viewed as a direct result of pollution. And because Bangladesh had little industrial pollution in those days, he was told by politicians and policymakers: "Why the need for environmental policy research?" That started to change, he says, with the floods of 1987–88 when they learned that natural causes alone had not caused the flooding. This led to a big debate about possible causes, including deforestation, and ultimately the human activities discussed at Rio.

Rio was also a turning point for biologist Cristián Samper, founder of the Alexander von Humboldt Biological Resources Research Institute in Bogotá, Colombia. "Rio is why we decided to establish a new national institute as a joint venture between the government, universities, non-governmental groups and the private sector," he explains. The Humboldt institute was set up in 1995 to foster scientific research in support of environmental policy.

Today, these and other institutes owe their existence to fiercely driven individuals — each fired up with a desire to harness the best available knowledge to solve their countries' environment and development problems. The success stories include Huq's centre in Dhaka, Samper's institute in Bogotá, the African Centre for Technology Studies (ACTS) in Nairobi, Kenya, and the Sustainable Development Policy Institute (SDPI) in Islamabad, Pakistan.

The founders of these four institutions were all young — from 29 to 43 years in age. But they weren't idealistic hotheads unprepared for the realities of building independent science-policy institutions in Africa, Asia and Latin America. They had all received postgraduate education in the United Kingdom or the United States. They were also well-connected at home and abroad, and had good access to funding.

> "The Rio meeting acted as a wake-up call to developing countries." — Saleemul Huq

They also needed resilience in the face of scepticism from international donors, from bureaucrats unable to see how good science could make for better policy and from scientific peers who were fearful of politics (especially those living in dictatorial regimes). Two decades later, their institutes are still in business — although, in every case, the founders have moved on to high-status positions elsewhere. As a model of sustainable development the attendees at Rio could hardly have wished for more.

Launch fund

The Rio meeting was also a major event in the life of Kenyan-born Calestous Juma, now a professor at Harvard University's John F. Kennedy School of Government in Cambridge. Back then, he was working as an adviser to the team drafting the text for the United Nations convention on biodiversity. He saw first-hand the interplay of science and politics as nations argued, horse-traded and made compromises to agree a final text in Rio. The experience reinforced his wish for Africa to join other countries in making policies on the basis of evidence.

After gaining a PhD in science policy at the University of Sussex, UK, Juma had returned to Kenya in 1987, when the country was under the one-party rule of President Daniel Arap Moi. "The original idea was to work with existing centres," Juma recalls, "but this turned out to be too difficult." He was forced to create a new institution to enable true cross-disciplinary policy research.

Today, that institution — ACTS — is one of Africa's leading research institutions and a source of independent advice to governments and international institutions on biotechnology and agriculture. But 20 years ago, scepticism among international donors meant that the centre began life in its founder's spare bedroom.

The launch funds came from the remainder of Juma's PhD scholarship, a US$2,500 donation from the Mennonite community of Nairobi and a $50,000 grant from the Ford Foundation. "The Mennonites were my first real sponsors," says Juma. "They have a reputation for being against the modern world, but what they oppose is consumption and they value innovation and creativity." Reasons enough, it seems, to support ACTS.

"Almost everyone else said that the idea would not fly," he recalls. I was told that policymakers don't read anything, so there was no reason to write for them. I was also told that there was no precedent for what I was doing in Kenya and that the political atmosphere at the time was too hostile — that I could go to prison, even."

His disappointment at the hands of international agencies was shared by other founders. In Bangladesh, Huq says that the agencies would have been more receptive if they had said: 'We want to provide running water in rural villages.' "They were less interested in helping developing countries to think for themselves," explains Huq. The prevailing practice at the time was that donors would hire expensive consultants to set the research framework. "Our job as scientists in developing countries would have been to implement their vision," he says.

Huq was fortunate in that one of his earliest supporters was his PhD supervisor, the ecologist Gordon Conway, now chief scientific adviser at Britain's Department for International Development. With Conway behind the idea, Huq was able to secure the backing of the Ford Foundation.

Today, the Bangladesh centre is one of Asia's leading environmental policy think tanks, advising governments and corporations on sustainable development, but Huq

says that he will never forget the uncertainty of those early days. "We had 40 or 50 families who depended on us. Sometimes I would sit in the office late into the night figuring out how people would be paid."

Former US economics professor, Tariq Banuri, found it easier to attract the attention of foreign donors. As founding director of Pakistan's Sustainable Development Policy Institute (SDPI), he says, "The vision was to create a trans-disciplinary research institution that was close to policy. In the late 1980s, such an institution would have been rare even in the developed West." But he was able to secure the backing of the governments of Canada, Norway and Switzerland, in part because the 1992 launch coincided with Pakistani thinking on an environment action plan. This helped to reassure donors that the SDPI would have some support from the state and was less of a freelance operation.

Banuri also had the backing of Aban Kabraji, a Pakistani conservationist who is a senior executive with the World Conservation Union (IUCN) and a well respected figure. "We did have a funding shortfall for a six month period, during which many of us worked unpaid, but that was not to be repeated," Banuri recalls.

Academic freedom

But friends in high places cannot alter one reality on the ground: a shallow pool of research talent in developing nations. Policy institutions need researchers who have an unusual blend of skills: in-depth knowledge of one or more scientific disciplines, along with knowledge or experience of how this applies in the policy arena.

Developed countries have many opportunities for academics to hone such skills. But in the developing world, public universities have less experience of working with governments, of collaborating across disciplines or of working with other non-academic institutions. It is because of this that most of the founders decided to operate independently of public universities.

In Pakistan, Banuri explains, "university staff were poorly paid and seemed unhappy and depressed. This was not the kind of environment I wanted for something new and innovative." Banuri also wanted the freedom that independence from the state provides. This freedom proved crucial in the case of a 2002 research project to survey public attitudes to Pakistan's nuclear development. "This project is unlikely to have happened had SDPI been linked to a public university," says project leader Haider Nizamani of the University of British Columbia. The survey's findings are due to be published in early 2008.

However, Banuri now thinks that, with hindsight, the decision to go independent was perhaps a mistake. "It would have been better for us to find a university, draw on its resources, but also help to build its capacity." Without building policy research capacity in universities, his thinking goes, independent institutions will have less talent to draw on.

Huq agrees that being based in a university would have been better in the long run. The Bangladesh education system never used to produce independent thinkers: "You could get a masters' degree by memorizing a textbook." But he adds that "things have changed in the past few years as Bangladesh higher education has opened up to private competition, which is raising the quality of graduates."

Instant impact

Once up and running, all four institutions found themselves much in demand from policymakers. As there is much less competition for the ear of politicians in these regions than in the developed world, the work of new institutions gets noticed and can have an impact more quickly. "The Humboldt Institute became a major force in science and environmental policy in Colombia in just a couple of years," Samper recalls. "We helped to create the country's first environmental act and had the environment minister chair our board." In Nairobi, ACTS helped to draft Kenya's first industrial property law in 1989, leading to the creation of the country's patent office. And in Dhaka, the Bangladesh centre helped to create a new environment ministry for the country and to write the country's first environmental action plan.

But Sunita Narain, who heads the Centre for Science and Environment in Delhi, cautions that having close ties to government carries risks as well as rewards. Her centre has a policy of not doing contract-research for anyone, and has taken a more activist role. Because India has long had a more stable democracy, unlike the other four countries, think tanks that combine policy and research are more common. Still, she says it is crucial for all institutions in the developing world to evolve in response to changing times. Now that environmental

CALESTOUS JUMA

Founder, African Centre for Technology Studies

Born: Port Victoria, Kenya, 1953
PhD: University of Sussex, UK (Science policy), 1987
Centre founded: Kenya, 1988
Current job: Professor of international development at Harvard University

What was the best advice you were given?
I went to see Maurice Strong (founding Executive Director of the United Nations Environment Programme) and told him that I wanted to start small and scale-up later. He told me that things that start small always stay that way. "Don't be afraid of having a big vision," he said.

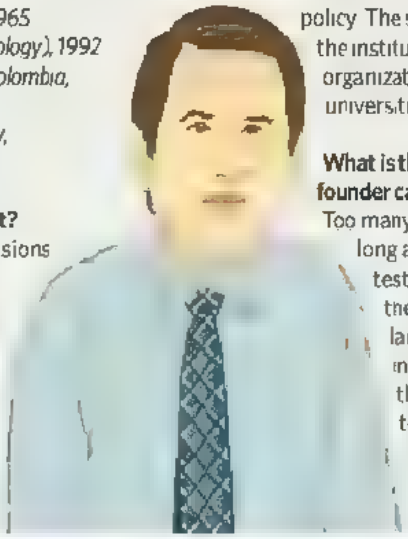
Would you do it all again?
I am in the process of creating a college in my home-town of Lake Victoria. The idea is to use the best available knowledge to create sustainable-development solutions for the area's people and ecology. I am too old to run it, but will act as a mentor to whoever takes it on.

CRISTIÁN SAMPER

Founder, Alexander von Humboldt Institute

Born: San José, Costa Rica, 1965
PhD: Harvard University (Biology), 1992
Institute founded: Bogotá, Colombia, 1995
Current job: Acting Secretary, Smithsonian Institution

What was the hardest part?
Overcoming the apprehensions of universities. Some felt that there was no need for another research institution. We had to explain this was not designed to duplicate work, but serve as a boundary institution between science and policy. The solution was to establish the institute as an umbrella organization and invite the universities to become members.



What is the worst mistake a founder can make?
Too many founders hang on for too long and never let go. The real test of a founder is whether they can let go and have laid the foundations for an institution to thrive without them. I have always tried to move on when things are going very well, but that is often when you are having the most fun.

issues have become mainstream, for example, think tanks need to find a niche to maintain their impact.

International partners, too, bring some risks. Each of the founders wanted international contacts but without turning their institution into a contract research centre for rich clients. For this reason, both the Bangladesh and Pakistan centres avoided bidding for lucrative overseas government contracts. But this was a form of research they were not always able to avoid, particularly when cashflow from other projects was tight.

Three of the centres formed a support network with other international partners. In Colombia, Samper says that such collaboration was seen as less of a priority. "I did suggest this to our board, but they took a different view and felt we already had the world's top expertise in Colombia," he says. But he doesn't think the centre's work suffered: "I would say that the best lessons and experience came from other developing countries."

Social scientists have a phrase to describe what happens when the founder of an institution refuses to hand over leadership to a new generation, sometimes in the mistaken belief that the organization will collapse without them. They call it 'founder syndrome'.

TARIQ J. BANURI

Founder, Sustainable Development Policy Institute

Born: Peshawar, Pakistan, 1949
PhD: Harvard University (Economics), 1986
Institute founded: Islamabad, 1992
Current job: Senior Fellow, Stockholm Environment Institute

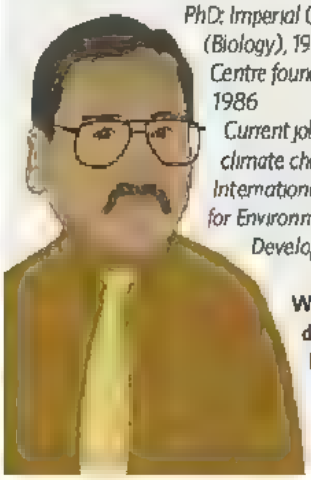
What would you do differently if given a second chance?
I would have been more 'journalistic'. Think-tanks that take a journalistic approach seem in hindsight to have been more influential. Looking back, I now wish we had found more young people with energy and dynamism who wanted to make a difference. Many of the big names we recruited turned out to be useless, whereas many of the bright young things that we hired are now the stars of tomorrow.

Would you do it all again?
Probably not. I have had offers to return to Pakistan, but my priorities are now more international, especially global climate change and helping to ensure that developing countries get a fair deal.

SALEEMUL HUQ

Founder, Bangladesh Centre for Advanced Studies

Born: Karachi, Pakistan, 1952
PhD: Imperial College, UK (Biology), 1978
Centre founded: Dhaka, 1986
Current job: Head, climate change group, International Institute for Environment and Development, London



What were you doing in 1986?
I had returned to Bangladesh to become an assistant professor at the University of Dhaka. It didn't suit me at all. It was bureaucratic: promotions were based on length of service, and the environment did not reward excellence or good ideas. So I got together with others who had also obtained PhDs abroad and we set up the Bangladesh Centre for Advanced Studies.

Would you do it all again?
Yes. In fact, I am planning to return to Bangladesh to establish an International Centre for Climate Change Adaptation and Mitigation, which will be based at one of the new private universities. Bangladesh is well placed to lead the developing world in responding to climate change. This will be my next challenge.

In the developing world, one of the most notorious examples of founder syndrome is that of the late Thomas Odhiambo, founding director of the International Centre for Insect Physiology and Ecology in Nairobi. Odhiambo, a charismatic figure, friend and adviser to generals and presidents, led the institution from 1970 until 1994 when he was forced out by the institution's donors and by its governing body. Odhiambo was determined that the centre should follow his vision. But the donors had other ideas and, today, the centre's website carries no reference to its founder.

> "I was determined to avoid a situation where the founder sticks to his creation like a leech and refuses to let go."
> — Tariq Banuri

Letting go

Mindful of the dangers of clinging on, three of the founders established limits to their terms as directors. And all four organized the recruitment of a successor and then cleared their desks when their time was up. "I was determined to avoid a situation where the founder sticks to his creation like a leech and refuses to let go," Banuri says. He adds, "I wouldn't have minded an offer to sit on the board after I left, but I felt board members were too afraid that I would try to continue to run the organization."

Banuri and Juma have since opted to return to working at the coal-face of research and are no longer responsible for the day-to-day administration of a large institution. By contrast, Samper and Huq are still active in the running of large organizations or collaborations. Huq remains non-executive chair of the board of the Bangladesh centre, and Samper is the acting head of the Smithsonian Institution in Washington DC. These different choices are perhaps explained by the contrasting styles of the founders. Both Banuri and Juma hold strong opinions and are not afraid to share them, whereas Samper and Huq would prefer to build a consensus.

The founders offer similarly contrasting advice to anyone wanting to follow in their footsteps. As Juma puts it: "If you have a good idea, don't consult too many people, as they will try to put you off." Samper, though, says it is important to: "take your time to build a vision, focus on a few priorities and have the buy-in of key stakeholders." He recalls that the best advice came from his father, who founded several agricultural research institutions in Latin America. "He said I should set a clear vision, hire a really good team and make sure I step down at the right time. The last of these is one of the hardest things, but I think it is crucial."

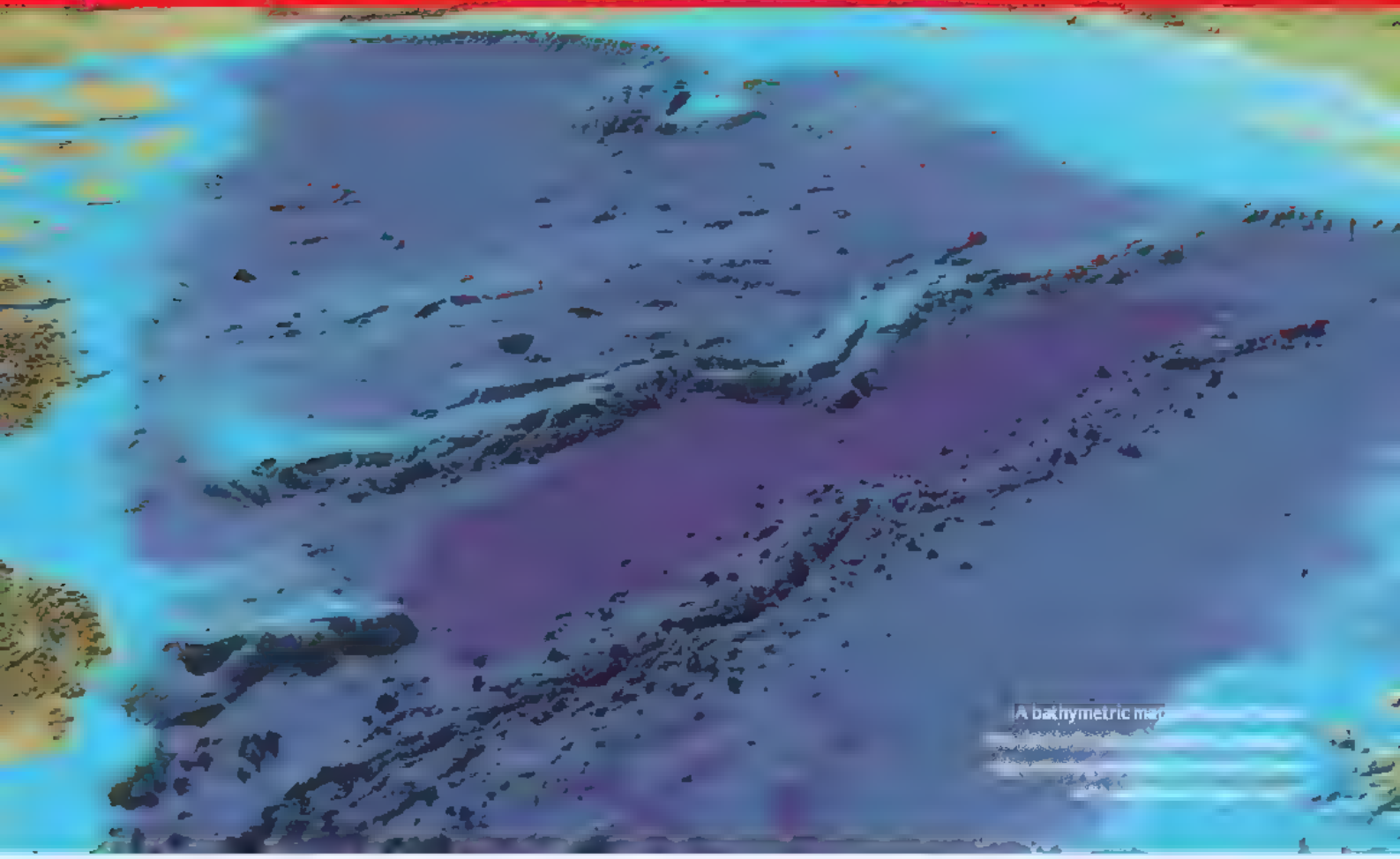
Not surprisingly, given their successful career paths since leaving the running of their institutes to others, each of the founders feels the experience was worthwhile, and will admit to few if any regrets. Some even say that they would be willing to start over again, in an advisory role if not an executive one. As mentor for his next project, Juma believes in finding donors who see the value of learning from mistakes: "This is critical for any new institution," he says, "but in the eyes of a donor, mistakes are to be punished."

And the most important lesson to pass on? There are no guarantees. In the same way that a research funding council cannot walk into a lab and demand the results of an experiment before it has even started, founding a new institute is a process of exploration without a certain outcome. In this sense, says Juma, "International development is much like basic research." ■

Ehsan Masood writes about science in developing countries.

See Editorial, page 1.

NATURE|Vol 451|3 January 2008



The next land rush

As countries race to file claims to areas of the sea floor before a United Nations deadline, geologists and geophysicists are getting caught up in the frenzy. **Daniel Cressey** reports.

Russia's twin Mir submersibles are perhaps the most-photographed explorers of the deep sea. Because they are manned, and can dive together to depths of 6,000 metres, film producers love sending them down for unprecedented footage. The 20-year-old Mirs have starred in a number of Hollywood adventures, including director James Cameron's exploration of the wreck of the *Titanic*.

So it is fitting that it was the flashy Mir-1 that planted a Russian flag on the sea floor at the North Pole last August, as part of a high-profile race to claim the riches of the Arctic Ocean. The stunt was, of course, purely symbolic — Russia does not own the sea floor at the North Pole, at least not yet. But the flag-planting reignited nationalistic debate about who has rights to what in the Arctic. And scientists play a crucial part in resolving those debates.

> "The map of the Arctic will change as a result of these actions."
> — Ronald Macnab

The latest area of interest is the Lomonosov ridge, a submerged rise about 1,800 kilometres long that runs from offshore Russia to offshore Greenland, crossing the geographic North Pole in the process. The ridge is also key to the ambitions of Russia, Denmark and Canada to lay claim to swaths of Arctic sea floor. The validity of their claims will be decided by a United Nations body, in accordance with the 1982 UN Convention on the Law of the Sea (UNCLOS). Under Article 76 of this treaty, a state can assert rights over the sea floor and its accompanying oil, gas and mineral wealth — beyond the standard 'exclusive economic zone' that extends to 200 nautical miles off the coast. For this to happen, the country must prove that the claimed area is a "natural prolongation" of its continental shelf (see 'How to split up the sea floor', overleaf).

Russia and Denmark both sent expeditions to the Lomonosov ridge last summer, but they are far from alone. A deadline of 2009 is approaching for some nations to stake claims under UNCLOS, and experts are poised for a rush of new applications worldwide. Most agree that the international body will eventually accept some of the claims. "There's no doubt in my mind the map of the Arctic will change as a result of these actions," says Ronald Macnab, a marine geophysicist formerly of the Geological Survey of Canada who now works as a consultant to those submitting claims.

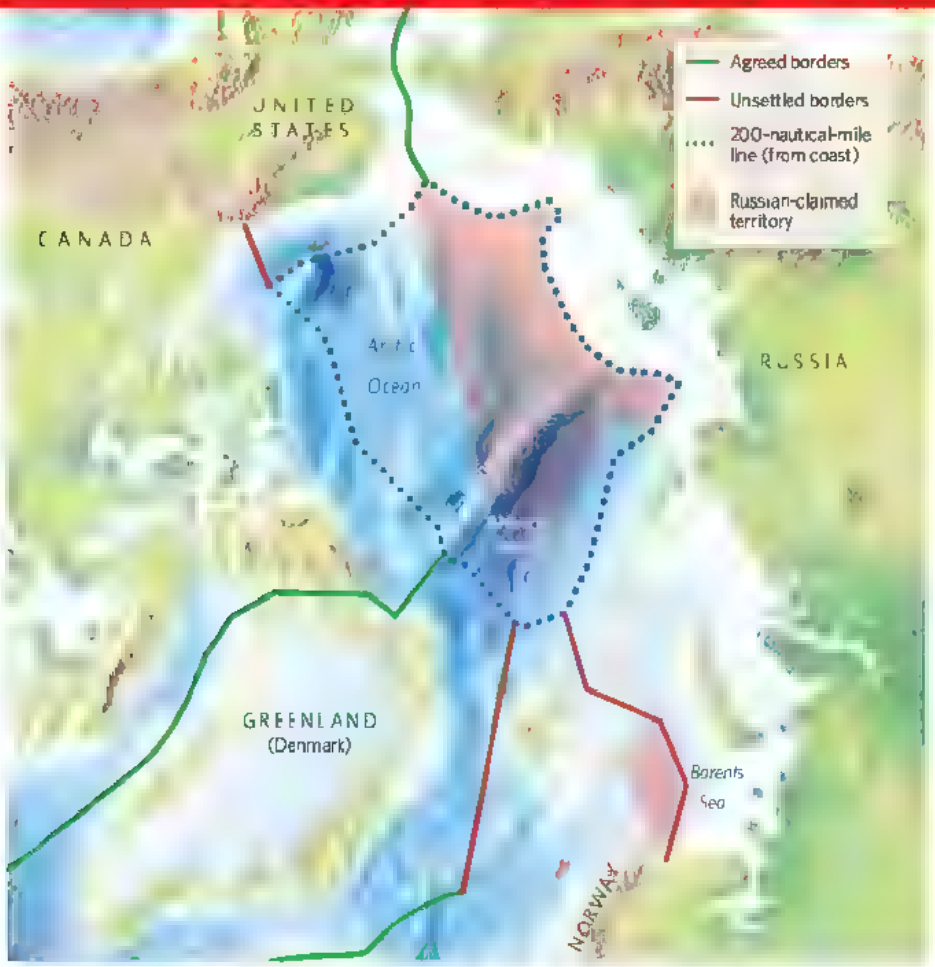
M. JAKOBSSON/IBCAO

But redrawing maps will take time. "There aren't going to be any easy claims under Article 76," says Lindsay Parson, an oceanographer at the University of Southampton, UK. In the process, geology and geophysics will have a significant role — as is already happening not only at the Lomonosov, but also in claims regarding the Bay of Biscay off France and Spain, the sea floor off New Zealand, and elsewhere.

Surveying the deep

The Lomonosov ridge, named after the eighteenth-century Russian scientist and writer Mikhail Lomonosov, is ground zero for the hottest claims. It may seem strange that the sea floor in the middle of the Arctic Ocean, miles from any land, can be considered a "natural extension" of Europe, Asia or North America. But the ridge's unique geology is behind this.

In the early 1960s, sea-floor mappers Bruce Heezen and Maurice Ewing recognized that



Up for grabs: Russia will not be the only nation to claim a share of the potential riches under the Arctic.

the mid-Atlantic ridge — the chain of underwater mountains that runs up the centre of the Atlantic Ocean, marking where new sea floor is born — extended into the Arctic, where it is known as the Gakkel ridge[1]. And in 1963, Canadian geologist J. Tuzo Wilson published a paper showing how the basin on the Siberian side of the Arctic Ocean could have opened up as the Gakkel produced new sea floor. That would have moved the Lomonosov ridge, which once formed a sliver along the edge of the Eurasian continent, over to run between Greenland and Russia[2]. In other words, the ridge was once part of Eurasia.

In 1991, researchers aboard the German icebreaker *Polarstern* and the Swedish icebreaker *Oden* set out to test this idea. Geologist Yngve Kristoffersen, of the University of Bergen, Norway, was part of the research team. "When we saw the first seismic survey I almost went through the roof," he remembers. "It was all there, just like a textbook." On the Eurasian side of the ridge lay half-grabens, features formed by fault rifting as the ridge pulled away from the continent. On the other side lay deep sediments. Kristoffersen and his colleagues estimated that the ridge subsided below sea level between 64 million and 56 million years ago[3].

In 2004, the first cores were drilled from the Lomonosov ridge, providing the first 'ground truth' for its geology and the history of the Arctic[4]. "Basically the bedrock is the same material from the Eurasian margin," says Kate Moran, an oceanographer at the University of Rhode Island, Kingston, who co-led the Arctic Coring Expedition. "There's full agreement that the ridge used to be connected to the Eurasian margin."

With the origin of the ridge not in doubt, the Russians must stake their claim on an interpretation of the brief passage in the UN rules, and must prove where exactly, if anywhere, the ridge is attached. "That is the difficult part," says Larry Mayer, director of the Center for Coastal and Ocean Mapping at the University of New Hampshire, Durham. "The issue is this demonstration of a natural prolongation of the land mass."

> **"There's full agreement that the ridge used to be connected to the Eurasian margin."**
> — Kate Moran

Moran says that some theories suggest that the ridge might have 'sheared' at the Russian end, meaning it is not really attached in the traditional sense. But such questions are difficult to answer without more data — hence the recent Russian and Danish expeditions.

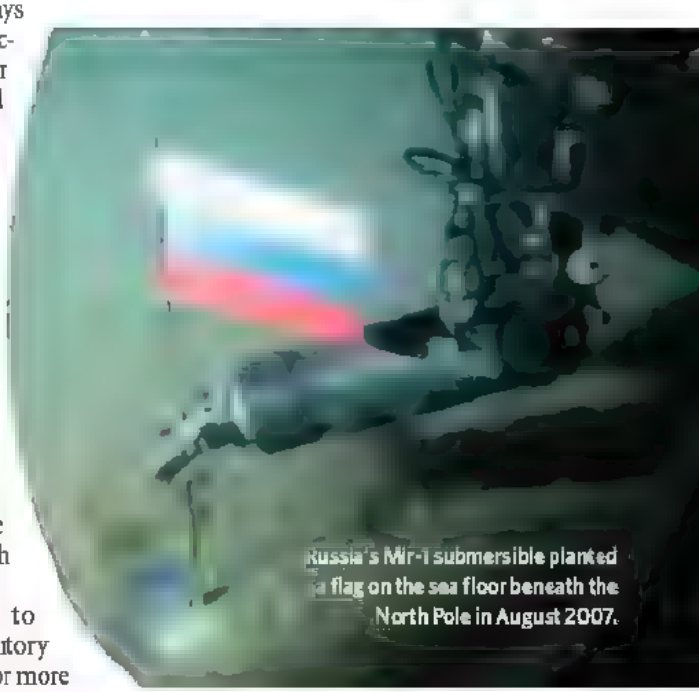
A 2001 Russian claim to UNCLOS for more Arctic territory was sent back with a request for more information. Exactly what was requested is not known, but this year's expedition included not only the Mir submersibles, which gathered water and sediment samples, but also aircraft running tests that included gravity surveys. Russian scientists are currently studying the data.

For their part, Denmark and Sweden sent two icebreakers as part of the Lomonosov Ridge off Greenland (LOMROG) 2007 expedition. The project ran into extraordinarily thick ice at the southern end of the ridge, but managed to undertake geological coring and oceanographic sampling, among other studies.

"We wanted to see change in composition going from the shelf onto the ridge," says Christian Marcussen, a senior adviser at the Geological Survey of Denmark and a principal investigator on the cruise. "What we're looking for is some kind of crustal continuation from the continent to the ridge."

But that may not necessarily hold sway with the UN committee deciding on the question of natural prolongation. "The geochemical affinity of rocks is not a defining factor in whether rocks are part of a nation's landmass," says Vaughan Stagpoole, a geologist at the Institute of Geological & Nuclear Sciences in Lower Hutt, New Zealand. "The key is in distinguishing such rocks from *in situ* 'deep ocean floor with its oceanic ridges', which does not form part of the natural prolongation of a state," he and his colleagues say in a statement to *Nature*.

Many of the LOMROG findings have not yet become public, as they are being prepared for publication in peer-review journals.



RTR RUSSIAN CHANNEL/AP

Russia's Mir-1 submersible planted a flag on the sea floor beneath the North Pole in August 2007.

or for submission to the UN commission. But a few details emerged last month at a meeting of the American Geophysical Union, in which co-investigator Martin Jakobsson, from Stockholm University in Sweden, reported features such as ice scours on the ridge, probably caused by giant icebergs dragging across the ridge surface. The Danish part of the team, led by Marcussen, is still working on data from their underwater surveys, which aimed to delineate the foot of the continental slope to better inform any claim Denmark may make under UNCLOS.

JPPA/PHOTOSHOT

The Union Jack flew above Rockall in 1955, but claims to the sea floor around the rock in the North Atlantic Ocean must come from geological proof.

Small country, big claim

For its part, New Zealand submitted a claim in 2006 that encompasses roughly 1.7 million square kilometres surrounding its islands. The claim is based on a host of complex features, including plateaux, ridges, seamounts and trenches, and also involves the first claim of a major active subduction margin. Some of the areas in the claim may overlap with other countries' entitlements (see map opposite), and New Zealand is in ongoing negotiations with Fiji and Tonga regarding this.

The New Zealand claim team had a budget of NZ$44 million (about US$30 million), underscoring the interest governments have in sea-floor claims. Under UNCLOS, nations get rights to exploit the oil, gas and mineral wealth in sea floor areas awarded to them. The team, however, says its survey was motivated by the desire to know what might be claimable under Article 76, as opposed to what might lie within that potential claim.

Other claims also seem to fall under the 'grab-it-now' mentality. Under UNCLOS, countries lose their rights to stake a claim once their deadline has passed. UNCLOS stipulates that countries have 10 years from the date they ratified the treaty to submit claims, with none coming before 2009. This gives deadlines of 2009 for Russia, 2013 for Canada and 2014 for Denmark — not a huge amount of time considering that the Arctic, for instance, is accessible to researchers for only a few months each summer.

> "The issue is this demonstration of a natural prolongation of the land mass."
> — Larry Mayer

In 2006, France, Spain, the United Kingdom and Ireland submitted a joint claim to a small area in the Bay of Biscay. The claim is based on a series of relatively small ridges that run out into the Atlantic, remnants from the period when the Iberian peninsula moved away from what is now France, creating the Bay of Biscay. "The process of ocean basin formation is not always a clean break," explains Parson, who is heavily involved in the UK claims. "Very often there's a lot of stretching and twisting that goes on. You can think of it as similar to breaking a biscuit — there are crumbs left over."

The Bay of Biscay claim is one of the less controversial under the UNCLOS process. "It's relatively small but the total area is about the same as the land area of Ireland — on that sort of scale it is significant," says Peter Croker, head of the team for Ireland's submission. "As submissions go it is relatively straightforward," says Croker, who also serves as a member of the UN Law of the Sea commission on the limits of the continental shelf. Subgroups within the commission decide on the merits of each claim, and they do not include any members who might have a stake in the claim being discussed.

More likely to be controversial are the expected claims around the hotly disputed 'island' of Rockall farther north in the Atlantic. The tiny, uninhabited outcrop that forms Rockall is frequently entirely swamped by waves and can itself support no claims, under UNCLOS, rocks that "cannot sustain human habitation or economic life of their own" cannot be used to claim the sea floor around them. But the sea floor surrounding Rockall could potentially be claimed as an extension of Ireland, the United Kingdom, Iceland or Denmark's Faroe Islands under the UNCLOS process.

The four countries have clashed before over Rockall. Ireland and the United Kingdom have agreed how to divide the continental shelf within their exclusive economic zones. But Iceland and the Faroes may also eventually claim part of the sea floor around Rockall — and the disputes are likely to rule out any previously-agreed joint submission as in the Bay of Biscay.

Other disputes may flare up farther south. The United Kingdom is expected to file a claim to the sea floor around what it calls the Falkland Islands — over which it fought a war in the early 1980s with Argentina, which claims the land as the Malvinas. Britain is also preparing a claim for sea bed off the coast of Antarctica, contiguous with its designated Antarctic territory on land. Australia and New Zealand have submitted similar claims off their Antarctic territories.

Flying blind

Controversies over claims are not helped by the secrecy of the UNCLOS process. Only summaries of submissions are published, and these need only contain a list of coordinates for the territory being claimed. "The commission operates under pretty serious rules of confidentiality," says Macnab. "We don't know how it has dealt with other submissions that may have similar circumstances. The states are flying blind."

The insistence on confidentiality is written into UNCLOS. "In an ideal world the whole process would be open," admits Croker. "The whole process is not transparent to the outside world. I understand why there's some frustration about it."

Inevitably, the criteria can lead to overlapping claims. For instance, Russia's Arctic claim in 2001 extended to, but not past, the North Pole, taking in the half of the Lomonosov

ridge that extends from the pole towards Russia. Claims are expected from Denmark and Canada regarding the part of the Lomonosov on their side of the pole; the countries would have to resolve between them where to put a boundary between their claims.

Indeed, the commission cannot even consider submissions on disputed areas without the permission of those involved in the dispute. Hence the joint submission for the Bay of Biscay, which involved researchers from all four countries on the survey team. If the claim is approved, the countries involved can then divide the land among themselves.

Redrawing the maps

Even so, scientists working in the area reject media and public statements that such claims amount to a selfish 'land grab'. "There is order in the oceans and that order is provided by the Law of the Sea," says Croker. "Terms such as 'land grab' are unfortunate to say the least."

But some researchers continue to stoke nationalistic fervour. Artur Chilingarov, who led the Russian expedition that planted the underwater flag, has been quoted as saying: "The Arctic is Russian. We must prove the North Pole is an extension of the Russian coastal shelf." Danish and Canadian politicians have made similar, if not quite so strident, statements.

In the end, though, the decisions that are made by UNCLOS will massively redraw sea-floor maps. Article 76 holds that the commission's recommendations will be "final and binding". Still, questions are being raised as to what exactly that means. "I've been at several meetings where people have questioned what it means, this 'final and binding'," says Macnab. "The legal people themselves aren't quite sure what the terms mean."

> "The Arctic is Russian. We must prove the North Pole is an extension of the Russian coastal shelf."
> — Artur Chilingarov

So all the sea floor wrangling may end up not even being permanent. "Although most countries have ratified the Law of the Sea," says Macnab, "there's nothing to say you wouldn't have some rogue government saying it didn't agree with some past decision and they were going to do things differently. It wouldn't surprise me to see states say 'we want to reopen'." In which case it is back to the drawing board. ■

Daniel Cressey is a reporter in *Nature*'s London offices.

How to split up the sea floor

In 1973 the Third United Nations Conference on the Law of the Sea was convened, in an attempt to bring some order to increasing unilateral declarations of 'rights' over the oceans and the inevitable conflict that ensued. When the conference ended nine years later, it gave birth to the UN Convention on the Law of the Sea. It has now been ratified by 155 countries, with a notable exception being the United States, which is caught up in congressional debates about national sovereignty.

The convention gave nations the rights over the continental shelf out to the limit of their exclusive economic zone, 200 nautical miles from shore. But if nations can prove other geological criteria, they can claim to either 350 miles from territorial waters, or 100 miles from the 2,500-metre depth.

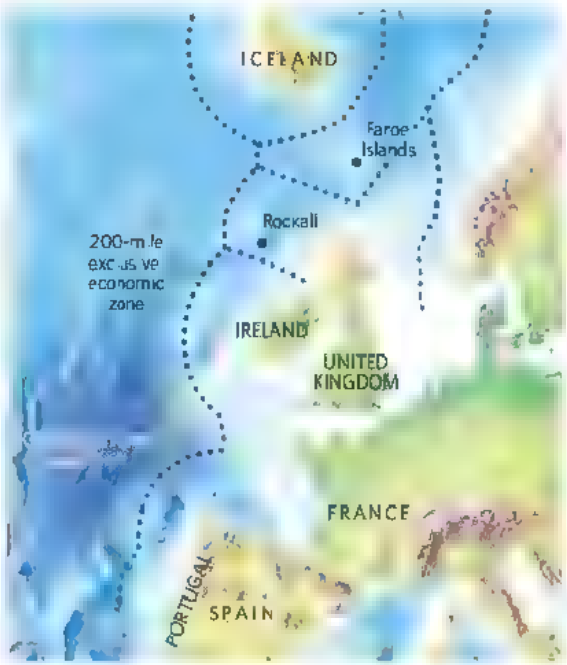
Article 76 itself, which lays out these criteria, is just 600 words long — leading to much confusion, particularly on the issue of what constitutes a claimable 'natural prolongation' of a continental shelf. "Here the commission has to sort out some guidelines that people can follow," says Wilfried Jokat, a geophysicist at the Alfred Wegener Institute for Polar and Marine Research in Potsdam, Germany. "The article is too general about this."

Decisions on claims are made by a subcommittee of the 21-member commission on the limits of the continental shelf, staffed by scientific experts in the field. To date eight claims have been submitted:

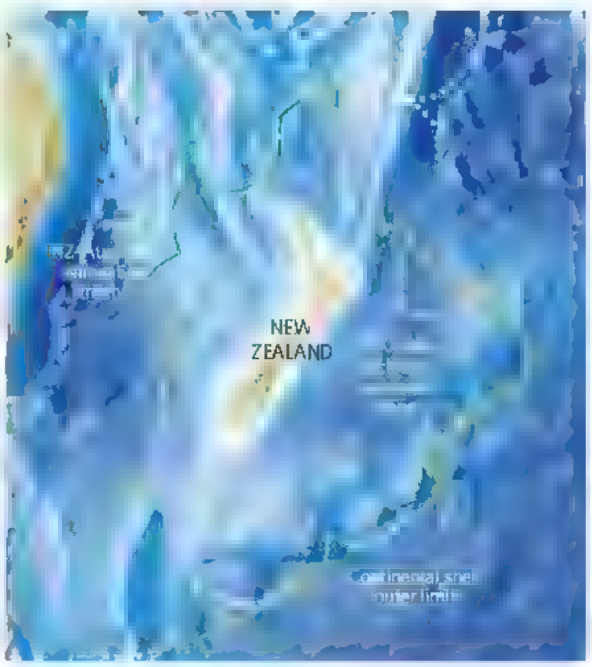
- Russian Federation: 2001
- Brazil: 2004
- Australia: 2004
- Ireland: 2005
- New Zealand: 2006
- Joint submission by France, Ireland, Spain and the United Kingdom: 2006
- Norway: 2006
- France: 2007

D.C.

1. Heezen, B. C. & Ewing, M. in *Geology of the Arctic* (ed. Raasch, G.) 622–642 (Univ. Toronto Press, Toronto, 1961).
2. Wilson, J. T. *Nature* **198**, 925–929 (1963).
3. Jokat, W., Oenzelmann-Neben, G., Kristoffersen, Y. & Rasmussen, T. M. *Geology* **20**, 887–890 (1992).
4. Moran, K. *et al. Nature* **441**, 601–605 (2006).



The Bay of Biscay (left-hand picture) has been subject to a joint extended-shelf claim by France, Spain, the United Kingdom and Ireland.



New Zealand (right-hand picture) covers only about 270,000 square kilometres, but its claim on areas beyond its exclusive economic zone could see it have access to 6 million square kilometres of sea floor.

Strategies and alliances needed to protect forest from palm-oil industry

SIR — Lian Pin Koh and David S. Wilcove propose in their Commentary 'Cashing in palm oil for conservation' (*Nature* **448**, 993–994; 2007) that non-governmental organizations (NGOs) should purchase and operate oil palm plantations, and that they should use the revenue generated to expand the network of private reserves in Indonesia and Malaysia.

Because this would delay the establishment of reserves in a landscape that is rapidly degrading, Reuben Clements and Mary Rose C. Posa in Correspondence ('Conservationists could slip up in palm-oil enterprise' *Nature* **448**, 403; 2007) advise using available funds to purchase the reserves directly instead.

However, both strategies focus on purchasing land for reserves. In doing this, they are putting themselves in competition with a palm-oil industry that is worth more than $4 billion in annual exports from Indonesia alone, according to the Food and Agriculture Organization (FAO, Rome, 2006).

Limited by their annual budget of only about $12 million, Indonesian NGOs are unlikely to be able to afford the cost of establishing sufficient reserves. Instead, what we suggest is that they combine a range of approaches to conservation, in order to maximize their influence through strategic alliances.

These include joining in with local communities who can sway development away from palm oil towards more sustainable land use, and working with carbon off-setters to redirect deforestation and carbon sequestration payments to areas of high conservation value. NGOs should engage the palm-oil industry through the Roundtable on Sustainable Palm Oil and encourage them to implement practices that improve the conservation value of their estate. The NGOs should also work to support an economically and ecologically sustainable timber industry.

Reserves should be purchased only if and when doing so is a more cost-effective means of conserving biodiversity than any of the available alternatives: that is, when resources are available, the benefits are substantial and the alternatives are limited.

Increasing consumer awareness of the impact of palm-oil production on biodiversity — particularly on orangutans (*Pongo pygmaeus*) — is another important step. A resultant drop in the demand, by people concerned about the environment, for biodiesel from palm oil could curb the industry's growth.

The NGOs involved must move on to foster relationships that simultaneously work both with and against the palm-oil industry to limit its impact.

Oscar Venter*, Erik Meijaard†, Kerrie Wilson‡
*The Ecology Centre, School of Integrative Biology, The University of Queensland, Brisbane, Queensland 4072, Australia
†The Nature Conservancy, Tropical Forest Initiative and Orangutan Conservation Services Program, Balikpapan, East Kalimantan 76100, Indonesia
‡The Nature Conservancy, 60 Leicester Street, Carlton, 3053, Australia

Illegal mining could revive Xinjiang's coalfield fires

SIR — Your *Nature News* story '50-year old fire put out' (doi:10.1038/news.2007.281) mentions that an underground coal fire in Xinjiang's Terak coalfield in China has been extinguished, as has another, near Urumqi, that had burnt for 130 years. However, we remain unconvinced that the Terak coalfield fire will not reignite.

As a result of more than three years' efforts by the Xinjiang Coalfield Fire-fighting Project Office, the coalfield fire in Liuhuanggou, near Urumqi, was put out in 2004. We have been detecting signs of revival since 2005, however, including sporadic heat anomalies in sub-zones and smoke escaping from surface fissures. Local blocking of the fires has caused a redistribution of ground stress, resulting in new fissures connecting with the surface. These fissures allow entry of oxygen and promote heat circulation. Meanwhile, mining in the vicinity may have disrupted fire-suppression measures, causing new fire spots to start up around the previous fire zone.

Since 2005, the Liuhuanggou coalfield roads have been levelled to facilitate fire-fighting operations. Coal that was previously hard to extract can now be easily mined, illegally, from the coal-seam outcrops just by stripping the soil overburden that was once covered for the purpose of firefighting.

Illegal mining may also have helped to revive the fire. In coalfields in Xiaolongkou and Xiaohuangshan, for example, where fires had been confirmed as extinguished in 2001 by the Chinese government, illegal mining activities have already caused the fires to revive.

The Chinese government should prohibit uncontrolled mining activities and maintain long-term monitoring in and around the extinguished fire zones to prevent the fires from reigniting.

Maohua Zhong, Tairan Fu
China Academy of Safety Science and Technology, State Administration of Work Safety, No. 17, Huixin Xijie, Chaoyang District, Beijing 100029, People's Republic of China

Slow development impedes the uptake of diagnostics

SIR — Two conclusions in your Business story 'Missing the mark' (*Nature* **449**, 770–771; 2007) about the usefulness of cancer biomarkers should evoke a response from translational researchers and clinicians.

First, you say that the overall impact of early bladder-cancer detection on patient survival rates may be relatively small because surgery remains the treatment of choice. This may be why survival from bladder cancer has barely changed during the past two decades. more accurate early-detection tools, such as biomarker tests, are needed. Since the 1980s, the American Cancer Society has issued guidelines and recommended several early-detection tests because of their clinical benefits. These have led to an increased likelihood of complete tumour removal and therefore of a better outcome and reduced costs. Public–private partnerships are therefore expediting the development of biomarker diagnostic tools.

Second, you say that, in the absence of new drug therapies, "there isn't a huge incentive for doctors and the health-care insurers that pay for most medical services in the United States to buy the tests". But this is contrary to established fact. For example, DNA-based testing for human papilloma virus to augment or replace pap smears is widely accepted and reimbursed. *Her-2/neu* gene testing is necessary for breast-cancer patients undergoing treatment with Herceptin. The US Food and Drug Administration's approval in 2004 of EGFR-pharmDx (which detects colorectal cancers expressing epidermal growth factor receptors) for treatment with the cancer drug Erbitux led to rapid uptake, with reimbursement by Medicare and private insurers in the United States.

The problem lies not with physician uptake and reimbursement, but with the slow development and validation of accurate tests providing useful information for early detection and treatment.

Chul-So Moon*, Eddy C. Agbo*, David Sidransky†
*Cangen Biotechnologies, Inc., 300 E. Lombard Street, Baltimore, Maryland 21202, USA
†Department of Otolaryngology, Johns Hopkins University School of Medicine, and Sidney Kimmel Comprehensive Cancer Center, Cancer Research Building II, 5N.03, 1550 Orleans Street, Baltimore, Maryland 21231, USA

Contributions to Correspondence may be submitted to correspondence@nature.com. Published contributions are edited. Science publishing issues of interest to authors are regularly featured at Nautilus (http://blogs.nature.com/nautilus), where we welcome comments and debate.

BOOKS & ARTS

Keeping pace with change

A textbook covering all aspects of evolution puts the spotlight on the molecular motor that drives it.

In batesian mimicry, the warning coloration of poisonous butterflies (top) is copied by harmless species (bottom) to improve their chances of survival.

COURTESY OF N. H. PATEL

Evolution
by Nicholas H. Barton, Derek E. G. Briggs, Jonathan A. Eisen, David B. Goldstein & Nipam H. Patel
Cold Spring Harbor Laboratory Press
2007. 833 pp. $100, £39.99

Daniel Hartl

As a young man I attended an evening party at the Cold Spring Harbor Laboratory in New York. Max Delbrück approached me and asked what I was interested in. Awed by the unexpected attention from so famous a scientist, I stammered something about genetic variation in natural populations and how, through time, this becomes transformed into morphological and other differences between species. As I began my second sentence, he interrupted to say, "Young man, you are wasting your time," and abruptly walked away. I brooded on this for a while — quite a while.

What Delbrück did not foresee (and nor did I) was that during the next 30 years molecular and cellular biology would flourish, and that in one of the great scientific revolutions in history these fields would spin off a succession of powerful new experimental techniques augmented by automation and computational power. Evolutionary biology prospered from these advances, and the field as I knew it then was a mere preamble to what it is today. Every branch of evolutionary study has been transformed and invigorated, and some branches have been created anew — for example, 'evo-devo' (the evolution of developmental mechanisms).

Textbooks in evolutionary biology have generally kept pace with these changes and several excellent books are available. This new one by Barton and colleagues is among the best. The production quality is superb in layout, composition, typesetting, colour palette, illustrations and gorgeous half tones; and the writing is excellent, as one might expect from such a stellar cast of experts in population genetics, palaeontology, human genetics, bacterial genomics and developmental biology (respectively).

The book is in four parts. The first is a history of evolutionary thinking and evidence for the evolutionary process, which clarifies common misconceptions about evolution and rebuts 'intelligent design'. The latter is unfortunately necessary in the United States, where people who think that space aliens have landed on Earth outnumber those who believe in the darwinian theory of human evolution by about 3:1.

Part I also includes an excellent introduction to molecular biology, although I suspect that much of this duplicates what most students already know. Part II, on the origin and diversification of life, is up to date with discussions on the last universal common ancestor, as well as being an outstanding introduction to evo-devo. Part III comprises about half the book and deals with the genetic mechanisms of evolution, including speciation, in a treatment that is fresh, thorough and professional. Subtle concepts, including Fisher's geometrical theory of adaptation and the coalescent, are clearly described with minimal mathematics. The final section is devoted to human diversity and evolution, and includes an engaging discussion of human nature.

This book may not fit every instructor's needs. Some may prefer a different balance of origin, diversity, molecular evolution, population genetics and human evolution, or they may need a textbook written at a different level. But every instructor should examine this book and make an individual decision.

The absence of end-of-chapter problems is a surprise. They are to be posted on the web soon, apparently. The web feature eliminates a lot of chapter-end clutter, but it will work only if students are motivated enough to access the problem sets and extra material online. Students who learn only the facts, but not how to use them or integrate them, will surely be wasting their time, no matter what their interests. ■

Daniel L. Hartl is Higgins professor in the Department of Organismic and Evolutionary Biology at Harvard University, Cambridge, Massachusetts 02138, USA.

Split world

Decoherence and the Quantum-to-Classical Transition
by Maximilian Schlosshauer
Springer: 2007. 416 pp. £54

Anton Zeilinger

Elementary particles, such as photons, electrons and neutrons inhabit a quantum world, where they may exist in a superposition between two or more locations at the same time. They can be entangled over large distances, or several of their properties may be undefined. In our everyday world, such phenomena are not evident — computers, cars and people are always at well-defined locations. And the properties of one are independent of what is done to another one at a distant location. Why do we not see quantum phenomena in macroscopic objects?

The term 'decoherence' describes how quantum superposition is lost as a result of entanglement between an environment and the internal or external degrees of freedom of the system. Whether or not this can explain the emergence of a classical world is a matter of philosophical debate. Nonetheless, the physics of decoherence is interesting. Maximilian Schlosshauer gives us a thorough and readable representation of today's understanding of the topic in *Decoherence and the Quantum-to-Classical Transition*.

The book gives an excellent overview of the various theoretical approaches to the physics that leads to decoherence. A particular strength is that it includes accounts of several experiments demonstrating the decoherence mechanism in detail, and shows how the results might predict experimental developments with quantum systems that become larger and more complex.

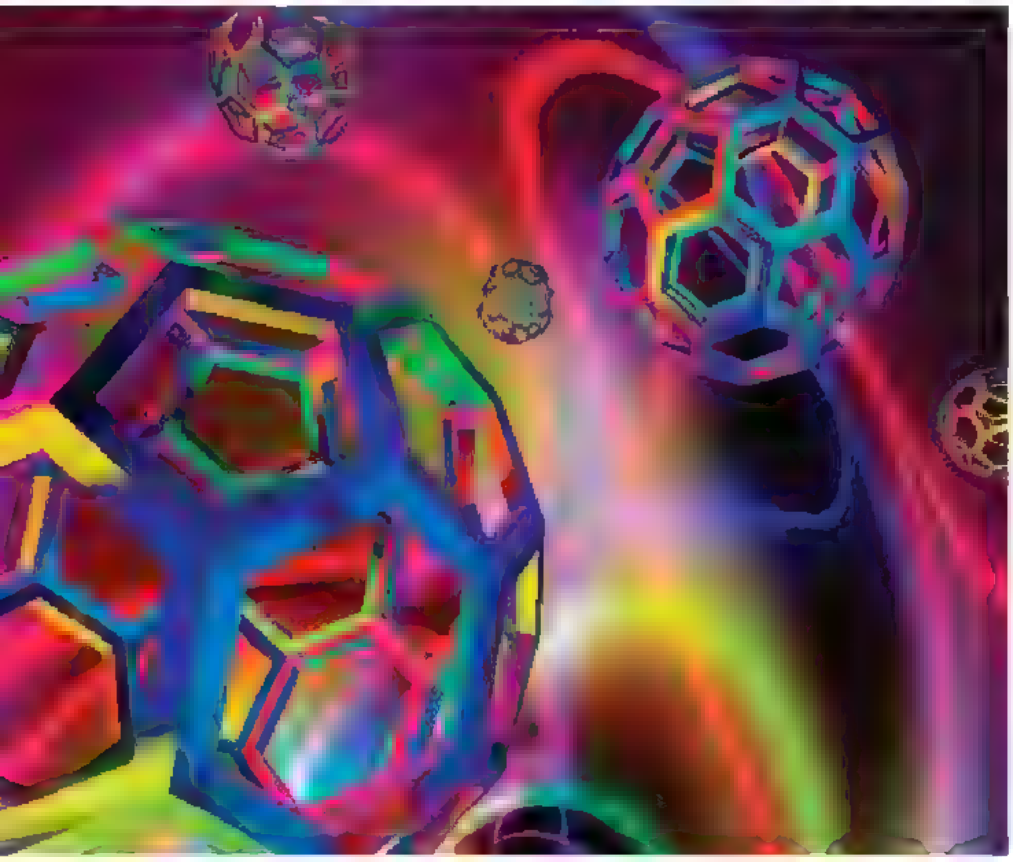
Schlosshauer also discusses various approaches to the fundamental and conceptual understanding of the nature of quantum states, and therefore of decoherence. Although he maintains a neutral position, I have the impression that he is taking the notion of quantum states in an ontological rather than an epistemological sense. For example (as is well known from early quantum mechanics and is now supported by experiment), there is no fixed boundary between the classical and the quantum world. The same object can behave as a quantum system in one situation, for example when it is isolated from the environment, or as a classical system in another.

Fullerenes and even biological macromolecules are typical examples, showing quantum interference in two-slit experiments whereas they can be seen in a tunnelling electron microscope, for instance, at classically well-defined locations. This shifting boundary is confirmed by the decoherence mechanism. But to argue that this is evidence against the Copenhagen interpretation, as the author does, is unjustified: the Copenhagen interpretation itself says that whether an object is classical or quantum is a function of the chosen experimental set up.

Decoherence is, to follow physicist John Bell, for all practical purposes sufficient to describe the loss of quantum features for large systems. There are still unanswered questions. It is well known, which Schlosshauer also stresses, that the interference terms never strictly vanish, so decoherence can tell us only that the interference terms disappear effectively but not rigorously. Even after accepting that approximation, we are still left with the system represented as a mixture of various possibilities, like being in two places at once. In the classical world, we know that the system is always at this place or at that place. To explain the two as equivalent is again, for all practical purposes, sufficient. Yet it involves, as Bell points out, another interpretive leap.

An author index and more discussion of the early history of decoherence would have been useful, but the book is an important resource for anyone interested in decoherence. It is very well written and it will contribute to further conceptual and theoretical development and to new experiments, extending the boundary of the quantum domain — maybe even into the macroscopic world. ■

Anton Zeilinger is professor of physics at the University of Vienna and the Austrian Academy of Sciences in Vienna, Austria.



G. KJELLENBERG, THE STOCK COLLECTION/GETTY IMAGES

Double life: these carbon-60 fullerenes can behave as either a quantum or a classical system.

Dendrites
edited by G. Stuart, N. Spruston and Michael Häusser
(Oxford Univ. Press: 2007. 2nd edition. £42.95, $79.50)

Dendrites are extensions of the cell body of the neuron, receiving synaptic input from thousands of other neurons and shaping and integrating these signals. This book, which is a collection of informative essays by prominent neuroscientists, captures the resurgence of interest in these structures, the intricacies of their function, and the contribution they make to mental processes.

The second edition follows eight years after the first. Chapters have been extensively revised or rewritten, and six of the 20 are new — reflecting the vigorous expansion of the topic into new areas. A multidisciplinary approach underpins the search for answers to the many questions posed by dendrites and gives insight into their development and their electrical, chemical and computational properties.

Each chapter is lavishly referenced and set out under concise subheadings, the colour illustrations, a staple of any worthwhile learning aid, are thoughtfully presented. There is a foreword by Nobel laureate Bert Sakmann. ■

OXFORD UNIV. PRESS

NATURAL HISTORY

Drawing conclusions

NAT. HIST. MUS., LONDON

For millennia, people have observed, recorded and documented the living world and attempted to make sense of it. *The Great Naturalists* (Thames & Hudson, 2007) charts the history of natural history through the lives of 40 such people over the past 2,000 years.

Alongside the expected names — Aristotle, Linnaeus, Darwin — this handsome volume celebrates the lives and works of many lesser-known figures, including Konrad Gessner, the sixteenth-century writer of *Historia Animalium* and *Historia Plantarum*, and Mary Anning, who discovered the first plesiosaur.

Four sections — 'The Ancients', 'The Renaissance', 'The Enlightenment' and 'The 19th Century' — all beautifully illustrated, often by the naturalists, show how the desire for striking images collided with the need for accurate documentation. Pictured is Georges Cuvier's giant ground sloth (*Megatherium*) skeleton. ■

Jenny Meyer

The greening of genomics

Functional Plant Genomics
edited by J. F. Morot-Gaudry, P. Lea & J. F. Briat
Science Publishers: 2007. 699 pp. £69.88, $119.50

Andrew H. Paterson

A global investment in plant genomics is under way, thanks to increasing recognition of the services provided by higher plants to ecosystems. These plants fix the greenhouse gas carbon dioxide, enrich soil constituents and are valuable as a source of food, fuel, fibres and medicines.

We already know the genome sequences of thale grass (*Arabidopsis thaliana*), rice, the poplar tree and the grapevine, and those of papaya, sorghum and others are in the pipeline. The sequences of most crop genomes will probably be in hand in the next decade. These will seed "the birth of a new plant biology", a concept that forms the focus of *Functional Plant Genomics* and promises to expose relationships between DNA sequence and botanical diversity.

The book — edited by former leaders of the plant biology department at INRA, the French agricultural research agency — exceeds the scope of its title. It reaches from the ancestry of genomics, through genome sequencing, annotation and functional dissection, to translation of functional information from botanical models into crop improvement. The section on structural genomics includes results from classical cytogenetics and renaturation kinetics research. The findings have shaped our understanding of genome organization, from cloning and sequencing to the computational techniques that convert sequences into information. Included are intuitive explanations of basic methodologies along with detailed coverage of knowledge and resources.

Complete or exhaustive sequencing is addressed in detail. The authors note that "the cost of a complete sequence ... in the future will have to be balanced against the [pro]portion of specific information", but coverage of advances in whole-genome shotgun (equated to "partial or draft-type") sequencing is brief.

The section on functional genomics considers large-scale approaches to quantifying gene function, as reflected by phenotype, expression of messenger RNA, and protein and metabolite levels. It also gives some attention to the statistical methods used to extract signals from these (often noisy) data. Metabolomics features prominently. There is a particularly interesting chapter on the application of *in silico* metabolic mapping to complement and accelerate the deduction of pathways that are followed by each molecule entering an organism.

A final short section on genomics and technology is an extension of metabolomics. It details metabolic fingerprinting for developmental, genetic and stress-induced variations, as well as the self-assembly (and potential for manipulation) of biopolymers that determine the quality and utility of plant products.

Functional Plant Genomics gives specific attention to the many manifestations of botanical diversity. It evaluates models that range from small genomes such as *A. thaliana*, with its experimental expediency and extensive infrastructure, to complex large genomes of plants such as sugarcane (dear to my heart), the world's number-one biofuel crop.

How can hard-won functional information best be applied to crop improvement through plant breeding? The book takes on this question by assessing DNA-marker types (including those derived from transposable elements), quantitative trait locus mapping, and approaches based on candidate genes and association with determinants of a trait. There is discussion of progress in utilization of genomics for breeding two very different cereals, maize (corn) and wheat. Maize uses cross-pollination and has two sets of chromosomes; wheat is self pollinating with six sets of chromosomes.

Minor errors and omissions notwithstanding, *Functional Plant Genomics* should broaden the perspective of researchers and postgraduate students on the role of genomics in the life sciences. Others will appreciate its glossary in what has become a fast-moving field pervaded by jargon. ■

Andrew H. Paterson is a distinguished research professor and director at the Plant Genome Mapping Laboratory, University of Georgia, Athens, Georgia 30602, USA.

Invertebrate Neurobiology
edited by Geoffrey North and Ralph J. Greenspan (Scion, Cold Spring Harbor Laboratory Press: 2007. £65.34, $135)

This monograph brings together two groups of scientists — molecular geneticists who study invertebrate organisms such as *Caenorhabditis elegans* and *Drosophila*, and classical ethological entomologists.

Invertebrate nervous systems are testament to the impressive range of solutions animals have evolved to live in all kinds of niches. The essays play on this diversity to illustrate molecular and cellular mechanisms of neural function, and higher-level circuits and systems.

Topics include 'Optic flow processing in the cockpit of the fly'; 'Insect walking', 'Memories of worms and flies: From gene to behavior', and 'Neuroendocrinology of eclosion'.

As Greenspan says, a lack of neuroanatomical homology with vertebrates may belie a deeper commonality in network architecture and in interactions among brain regions. The book assesses the universality of brain mechanisms and cognitive features, providing some useful pointers to what researchers studying vertebrate systems can learn from invertebrates. ■

CSHL PRESS

Ri The Royal Institution of Great Britain

nature

The Niche Prize

Could you create an arresting and inspiring image or installation that conveys a scientific idea in a highly novel way?

In spring 2008, the Ri will reopen its doors following a £20 million refurbishment. Here is your opportunity to fill a niche – both literally and metaphorically – for one year in this unique and iconic building.

Judges will include Susan Greenfield, Director of the Royal Institution, and Philip Campbell, Editor-in-Chief of Nature. This prize provides an unprecedented chance to become part of a key moment in the Royal Institution's 208-year history of celebrating science

Closing date 22 February 2008
An entry form and guidelines can be found at **www.rigb.org**

naturenews

Science news from a different angle

From daily science updates to investigative journalism, from community commentary to editorial opinions, *Nature* brings you the most in-depth science news coverage online.

Visit our new blog, *The Great Beyond*, for a comprehensive round-up and expert discussion of today's science news from around the world.

Subscribe to *Nature* and receive full access to *Nature News* as well as weekly issues of the most influential scientific journal in the world, in print and online.

www.nature.com/news

nature publishing group npg

NEWS & VIEWS

DOWN'S SYNDROME

Paradox of a tumour repressor

David W. Threadgill

Having three copies of chromosome 21 reduces the incidence of solid tumours in people with Down's syndrome. Studies in mice provide clues to why, and highlight a complex gene-function relationship.

Correlative epidemiological studies are powerful analytical tools that can provide insight into human health and disease. A case in point is the proposition[1] that the age-adjusted incidence of solid tumours is lower in individuals with Down's syndrome than in the general population. But correlative studies frequently lack a mechanistic foundation. And epidemiological studies occasionally reach conflicting conclusions — as has been the case with the link between Down's syndrome and solid tumours — leaving final verification to laboratory-based studies. Sussan *et al.*[2] (page 73 of this issue) used an innovative combination of mouse models to confirm a link between Down's syndrome and a lower incidence of solid tumours. The work offers an explanation for why having three, rather than the normal two, copies of chromosome 21, the hallmark of Down's syndrome in humans, provides some protection from solid tumours.

One mouse model of Down's syndrome that the authors studied is called Ts65Dn, and has been used extensively to study the biology of Down's syndrome[3]. These mice carry three copies of part of mouse chromosome 16, representing about half of the genes that are evolutionarily related to those carried on human chromosome 21. Moreover, Ts65Dn mice show features similar to those of individuals with Down's syndrome, including defects in the central nervous system and facial attributes. Sussan *et al.*[2] mated Ts65Dn mice with Apc^{Min} mice, an animal model of intestinal cancer[4]. They found that, compared with mice carrying two copies of mouse chromosome 16, the offspring of TS65Dn and Apc^{Min} mice, which carry three copies, developed significantly fewer intestinal tumours, and tumours that did develop were smaller.

Another mouse model that Sussan *et al.* examined (Ts1Rhr) recapitulates the minimal genetic alteration found in Down's syndrome, thus allowing the authors to narrow down most of the tumour-repressive effects observed with three copies of chromosome 21 to just 33 genes. A complementary mouse mutant, Ms1Rhr, which carries only a single copy of the same 33 genes, was also mated with Apc^{Min} mice. The resulting offspring, which carried a single copy of the 33 genes, showed a significant increase in tumour number compared with mice carrying two copies. This observation suggests a gene-dosage effect, in which the copy number of a particular gene correlates with the magnitude of its physiological effects. Consequently, the authors propose that the effect of three copies of chromosome 21 on tumour number is distinct from that of a typical tumour-suppressor gene, which affects tumour growth only by its presence or absence and not by its dosage. Rather, the effect of three copies of chromosome 21 is consistent with that of a tumour repressor.

Sussan and colleagues[2] further demonstrated that, of the 33 genes affected in Ts1Rhr and Ms1Rhr mice, the expression levels of only one gene, *Ets2*, correlate directly with its copy number and inversely with tumour number in Apc^{Min} mice. The authors confirmed that *Ets2* is the main tumour-repressor gene affected in Down's syndrome by specifically altering the number of this gene's copies in Ts1Rhr mice and also by investigating the effect of its dosage in Apc^{Min} mice.

This finding is surprising, because increased activity of the Ets2 protein, which regulates expression of a complex array of genes[5], has historically been linked to cancer promotion. For example, a contrast to the repressive effect of Est2 on tumour number in Apc^{Min} mice is that, in the PyMT mouse model of breast cancer, the normal two copies of *Ets2* are required in the stromal cells — which support cells

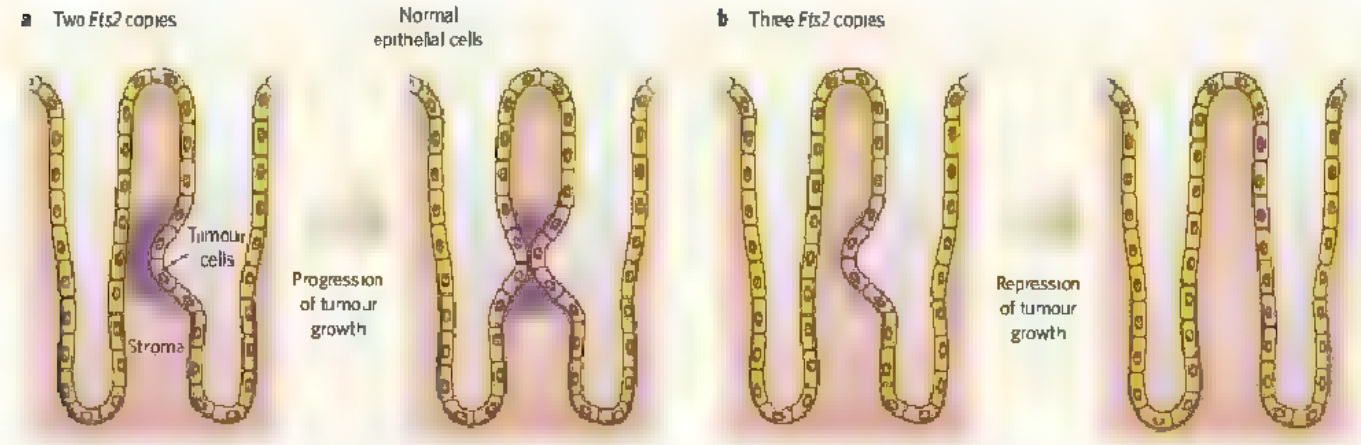


Figure 1 | The effect of *Ets2* copy number on intestinal tumour growth. On the basis of Sussan and colleagues' findings[2], a model linking *Ets2* dosage to early induction of polyclonal intestinal tumours can be proposed. **a**, In the presence of the usual two copies of *Ets2*, normal cells (yellow) and tumour cells respond to a cancer-inductive signal (red). This might induce alterations in the stroma, promoting growth of nearby cells and polyclonality which probably enhances the survival of early intestinal tumours. **b**, Three copies of *Ets2* in epithelial cells may reduce early tumour growth, limiting changes to the local stroma. A consequence would be reduced inductive activity on nearby cells, limiting the likelihood of tumour polyclonality and suppressing tumour survival.

50 & 100 YEARS AGO

50 YEARS AGO

What Man May Be: The Human Side of Science by George Russell Harrison — It has been estimated that each year sees the discovery of at least one million new scientific facts. Very few of us who are scientists have the capacity to digest more than a minute fraction of this feast, and to see the pattern to which it contributes. Yet we are vastly better off than the non-scientists, whose main contact with science is "through its slums, the half-world of such things as flying saucers and water dowsing" .. This is an excellent book to give to almost anyone who wants to understand that science has changed both the things we do, and the way we think. There is not a dull moment in it, and probably most of those who do read it will catch something of the infectious optimism that underlies each page. Onward and upward in the best of all possible worlds.

From *Nature* 4 January 1958

100 YEARS AGO

"The inheritance of 'acquired' characters" (*Sur la Transmissibilité de Charactères acquis*) by Eugene Rignano — A man of science to command general attention and interest must do two things; first, he must make interesting discoveries or profound generalisations; and secondly, he must do things at the right time. Darwin made his name because he fulfilled both conditions. Mendel died an unknown man because he did not fulfil the second. He was forty years too soon .. If it is possibly fatal to make discoveries too soon, it is certainly fatal to make them too late. It is therefore with a certain sense of weariness, mingled with surprise, that we note the appearance of a work on the transmission of acquired characters. The author of the book before us, who is an engineer interested in sociology, believes in the transmission of acquirements, and has invented a theory of centro-epigenesis to account for the phenomenon.

From *Nature* 2 January 1908.

within organs — for efficient tumour growth[6,7]. It seems, therefore, that Ets2 has context-dependent functions: in the Apc^{Min} mice with intestinal cancer it is a tumour repressor and in the PyMT mice with breast cancer it functions as a tumour promoter within the non-cancerous stromal cells. Such a non-cell autonomous function of Ets2 as a tumour promoter is consistent with observations[8] that Ets2 regulates the expression of genes within stromal cells to produce the extracellular matrix that is known to be essential for tumour growth and metastasis.

Taken together, the tumour-promoting and tumour-repressive activities of Ets2 may provide an intriguing explanation for the inverse correlation between *Ets2* copy number and intestinal tumour number in Apc^{Min} mice (Fig. 1). Previous studies have suggested that, in Apc^{Min} mice, early tumour cells have an inductive activity on nearby cells, which leads to polyclonality — tumours originating from more than one cell population[9]. The activity of Ets2, which can regulate stromal function as well as tumour growth, may contribute to this polyclonality and subsequent tumour survival. Although speculative, this hypothesis can be tested with animal models such as those used by Sussan and colleagues[2].

These authors' results undoubtedly provide insight into the contextual function of *Ets2*, but also reveal a paradox that will require further study. The contradictory activities of Ets2 predict a crucial point — individuals with Down's syndrome are at a lower risk of developing solid tumours, probably owing to the high Ets2 levels in their epithelial cells, but they might be at a greater risk of cancer metastasis. So therapeutic use of potential drugs with Ets2-like activity to reduce tumour incidence may have limited value, because a side effect of such drugs could be increased efficiency of metastasis[10]. Nonetheless, the findings of Sussan *et al.*[2] strongly warrant further investigation into whether natural variation in *Ets2* expression levels is associated with differential susceptibility to solid tumours. ■

David W. Threadgill is in the Department of Genetics, University of North Carolina at Chapel Hill, North Carolina 27599, USA.
e-mail: dwt@med.unc.edu

1. Record, R. G. & Smith, A. *Br. J. Prevent. Social Med.* **9**, 10–15 (1955)
2. Sussan, T. E., Yang, A., Li, F., Ostrowski, M. C. & Reeves, R. H. *Nature* **451**, 73–75 (2008)
3. Reeves, R. H. *et al. Nature Genet.* **11**, 177–184 (1995)
4. Moser, A. R., Pitot, H. C. & Dove, W. F. *Science* **247**, 322–324 (1990)
5. Seth, A. & Watson, D. K. *Eur. J. Cancer* **41**, 2462–2478 (2005)
6. Neznanov, N. *et al. Cancer Res.* **59**, 4242–4246 (1999)
7. Tynan, J. A., Wen, F., Muller, W. J. & Oshima, R. G. *Oncogene* **24**, 6870–6876 (2005)
8. Watabe, T. *et al. Int. J. Cancer* **77**, 128–137 (1998)
9. Halberg, R. B. & Dove, W. F. *Cell Cycle* **6**, 44–51 (2007)
10. Ito, Y., Takeda, T., Okada, M. & Matsuura, N. *Anticancer Res.* **22**, 1581–1584 (2002)

MAGNETISM

Freedom for the poles

Oleg Tchernyshyov

Magnetic poles always come in twos, a north and a south. That received wisdom has not stopped physicists from searching for 'monopoles' in accelerators and cosmic rays. Theory now indicates a better place to look.

Despite some tantalizing clues for their existence from the realms of quantum physics, magnetic monopoles — single magnetic poles without a partner — remain elusive after decades of searching. Do they exist at all in the real world? On page 42 of this issue[1], Castelnovo, Moessner and Sondhi argue yes: monopoles are alive and well in an exotic class of magnetic material known as spin ice[2].

An iron magnet has two poles, north and south: Earth's iron core makes it an extremely large example of the genre. These poles are positive and negative magnetic charges, acting as sources and sinks of the magnetic field. In general, magnetic interactions are very similar to electrical interactions: like poles repel and unlike attract, with a force inversely proportional to the square of their separation. But whereas positive and negative electric charges can exist independently, magnetic poles always seem to occur in pairs. Rather as the sorcerer's apprentice hacks his enchanted broom into pieces only for each to spring to life as a new whole broom, breaking a bar magnet in two yields smaller magnets, each with a north and a south pole, and an overall magnetic charge of zero.

This asymmetry extends to the subatomic level. Elementary particles can carry a positive or negative electric charge, but the magnetic charge is zero without exception. Yet theory offers some hints that single magnetic poles might exist in nature. In the 1930s, Paul Dirac showed that magnetic monopoles could explain the observed quantization of electric charge. Extensions of the standard model of particle physics include particles with magnetic charge.

One environment in which monopoles might pop up is crystalline solids. In a crystal at a low temperature, excitations above the ground state often behave like elementary particles: they

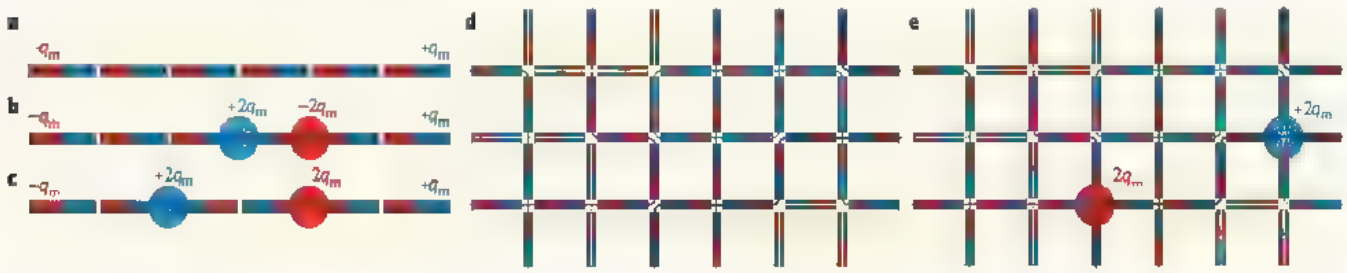


Figure 1 | Making monopoles. **a**, In the lowest-energy state, all the elements in a chain of magnetic dipoles point in the same direction: the north pole (magnetic charge $+q_m$) of one magnet touches the south pole (magnetic charge $-q_m$) of the next. The charges cancel out all the way along the string, except at the ends. **b**, Flipping one of the dipoles in the middle excites the chain out of its ground state, creating two magnetic charges $+2q_m$ and $-2q_m$. **c**, Each of these charges can be moved independently of the other by flipping a dipole next to it — they are free monopoles. **d**, Castelnovo *et al.*[1] study spin orientations in spin ice. Shown here is square spin ice, a two-dimensional variant that has been produced in an artificial form, as an array of nanoscale magnets[10]. Four magnetic poles meet at each point on the square lattice, and the energy is lowest when two are north poles and two are south poles. Spin ice in the ground state can be construed as a series of strings of magnetic dipoles embedded in a higher-dimensional lattice. **e**, Flipping dipoles on a single string (the black one) creates a pair of well-separated magnetic poles just as in one dimension.

carry a quantized amount of energy, momentum, electric charge and spin. In their theoretical study, Castelnovo *et al.* find the first instance of such an excitation with a non-zero magnetic charge. Under certain conditions, these magnets behave as a gas of independent magnetic poles. There is even a phase transition at which a thin vapour of these monopoles condenses into a dense liquid.

How a monopole can be created in a world of magnetic dipoles can be understood by considering a one-dimensional string made by laying tiny dipoles end to end. In this case, a single misaligned dipole gives rise to two independent magnetic charges that can be moved far apart, for the price of putting some energy into the system (Fig. 1a–c). The monopoles that arise are boundaries separating regions with perfectly aligned dipoles. These topological defects, known as domain walls, or 'kinks', have recently been studied in magnetic nanowires[3].

The emergence of free magnetic monopoles is an example of the phenomenon known as 'fractionalization' that the collective behaviour of many particles in a condensed-matter system is most effectively described in terms of fractions of the original particles. Fractionalization is often tied to topological defects[4] and is common in one-dimensional systems, such as the string already mentioned. The only confirmed case in two dimensions is the fractional quantum Hall effect, which occurs in a cold gas of electrons placed in a strong magnetic field[5]. Measurements of conductance[6] and electrical noise[7] in this system indicate the involvement of 'quasiparticles' with one-third of an electron's charge.

Castelnovo and colleagues provide the first example of fractionalization in a three-dimensional system. But how does the physics of free monopoles on a string survive in a higher-dimensional setting? The answer lies in the special nature of the ground states of the authors' chosen system, spin ice, which allows one-dimensional ideas to be transferred to two and three dimensions (Fig. 1d,e).

The monopoles in spin ice are magnetic analogues of electrically charged defects H_3O^+ and OH^- in water ice. The movement of these defects through water ice causes it to conduct electricity when an electric field (potential difference) is applied across it. Might it be possible to create a steady magnetic current in spin ice by placing it in a magnetic field? Unfortunately not. The motion of a kink alters the state of a string, making it impassable to the next magnetic charge. In water ice, a kink of a different flavour, known as a Bjerrum defect[8], repairs the damage done by the original defect. Because there is no analogue of Bjerrum defects in spin ice, magnetic monopoles are somewhat limited in their motion, and cannot sustain a direct magnetic current.

That still leaves the possibility of generating an alternating magnetic current, which would be interesting in its own right. In any case, learning how to move magnetic monopoles around would be a step towards technologies such as magnetic analogues of electric circuits and magnetic memories[9] operating on the atomic scale. ■

Oleg Tchernyshyov is in the Department of Physics and Astronomy, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA.
e-mail: olegt@jhu.edu

1. Castelnovo, C., Moessner, R. & Sondhi, S. L. *Nature* **451**, 42–45 (2008).
2. Bramwell, S. T. & Gingras, M. J. P. *Science* **294**, 1495–1501 (2001).
3. Saitoh, E., Miyajima, H., Yamaoka, T. & Tatara, G. *Nature* **432**, 203–206 (2004).
4. Goldstone, J. & Wilczek, F. *Phys. Rev. Lett.* **47**, 986–989 (1981).
5. Laughlin, R. B. *Phys. Rev. Lett.* **50**, 1395–1398 (1983).
6. Tsui, D. C., Stormer, H. L. & Gossard, A. C. *Phys. Rev. Lett.* **48**, 1559–1562 (1982).
7. de-Picciotto, R. *et al. Nature* **389**, 162–164 (1997).
8. Petrenko, V. F. & Whitworth, R. W. *Physics of Ice* 73–76 (Oxford Univ. Press, 1999).
9. Thomas, L. *et al. Nature* **443**, 197–200 (2006).
10. Wang, R. F. *et al. Nature* **439**, 303–306 (2006).

AQUACULTURE

The price of lice

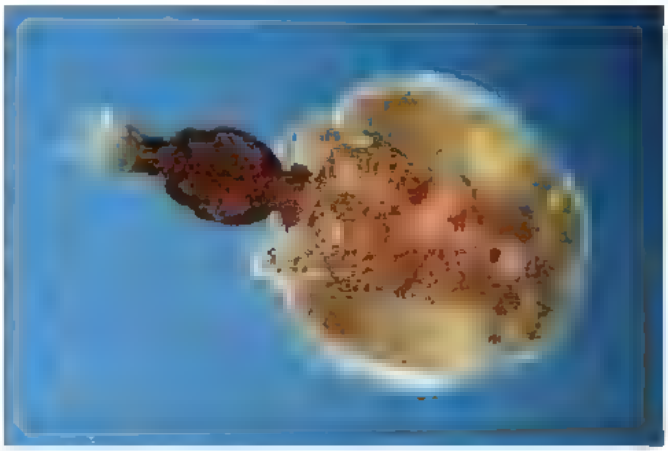
Andrew A. Rosenberg

Wild salmon stocks in Canadian coastal waters are being severely affected by parasites from fish farms. So intense are these infestations that some populations of salmon are at risk of extinction.

The global demand for fish is on the rise, and farmed sources are taking much of the strain — the catch of wild fish has levelled off, and may well be declining[1], but aquaculture production is expanding rapidly[2]. The ecological costs of that expansion can be heavy, however, as Krkošek *et al.* show in *Science*[3]. The message of their paper is that there are some serious issues that cannot be ignored if the expansion of aquaculture is to be productive rather than destructive.

Consumers can readily see the shift towards aquaculture, particularly for products such as farmed salmon, which has become a staple of supermarkets and restaurants in Europe and North America. Those buying fish will be aware of press reports of overfishing and resource depletion. Some may even look for eco-labels or carry a little card to guide them towards the purchase of sustainable products. As my colleague Carl Safina has said, "Give a man a fish and you have fed him for a day. Give a man a seafood choice card and you have made him impossible to dine with."

But aquaculture products tend to be subject to less public attention, even as issues



BLOOMWINE/HART.

Figure 1 | Salmon louse. This marine organism belongs to a group of crustaceans known as copepods. It feeds on the external surfaces of fish, and can eventually kill them.

ranging from habitat destruction to the effects of using wild fish to feed farmed stocks[4] become of greater concern. The emphasis of aquaculture development has, not surprisingly, been on increasing production, lowering costs and improving products. These needs of the industry have been well served by the science of fish farming. Unfortunately, however, research pointing out the environmental costs of production has been viewed as an attack on the industry, rather than as a challenge to be tackled and overcome.

Krkošek *et al.*[3] have provided new, empirical evidence of the environmental costs of the nearshore, net-pen aquaculture of salmon — the pending extinction of several populations of wild pink salmon, *Oncorhynchus gorbuscha*, on the coast of British Columbia. Until now, most research on the effects of net-pen aquaculture has revealed instances of certain consequences, such as the competition of wild fish with escaped farm stocks for spawning habitat, but not of effects at the population level. Krkošek and colleagues' analysis of 142 populations of pink salmon shows that wild stocks adjacent to fish farms have suffered dramatic increases in mortality of juvenile fish owing to infestation by sea lice, *Lepeophtheirus salmonis* (Fig. 1), and that most of the exposed populations are at risk of extinction within four salmon generations. It is the location of the salmon farms, compounded with the tendency of sea lice to proliferate near intensive farm facilities, that are cause for concern. Although farmed salmon can be treated to reduce sea-lice infestation, wild stocks have no such protection. As juvenile wild salmon emerge from rivers, their migration route runs a gauntlet of salmon aquaculture pens and, therefore, high levels of salmon lice.

The lesson of this analysis[3] is that neither fisheries science nor the aquaculture industry can be driven solely by the desire to increase production. Many aquaculture facilities are set in complex ecosystems, and influence the structure and function of those ecosystems. Policy-makers must ensure that the environmental costs are evaluated and monitored, and are factored into decisions about farm location and expansion. The argument that costs will be unfairly passed on to consumers rings hollow, because if such costs are not controlled, we the public eventually bear them in their entirety. In such a circumstance, there is little incentive for producers to reduce environmental impacts. The results of Krkošek and colleagues' study, and their warning that the terrible cost of extinction of wild salmon stocks is not far off, highlights that point.

Net-pen aquaculture is not just about salmon. As the industry expands into other species, such as cod, halibut and sablefish, the same concerns over the location of farms, disease and parasite transmission, and other impacts will certainly apply to these species too. It is vital to assess the potential environmental costs and to reduce them before the advent of large-scale farming of these species.

Can we design aquaculture systems that reduce ecosystem impacts, or eliminate some of them entirely? I think that we can, but not with a confrontational mentality. Overfishing of wild stocks is not a contrived problem, nor is it unsolvable: good management practices are often contentious and difficult to implement, but ultimately they can work. Similarly, the problems facing the aquaculture industry are not unsolvable, but denial that those problems exist will not provide answers. In the case of salmon lice, solutions include adhering to strict guidelines on the introduction and transfer of non-native fish, and siting of net pens away from areas where wild stock is vulnerable. Overall, the priority in aquaculture should be to anticipate any adverse environmental consequences and to tackle them at that stage, rather than struggle to recover after those consequences are already apparent. ■

Andrew A. Rosenberg is at the Institute for the Study of Earth, Oceans and Space, University of New Hampshire, Durham, New Hampshire 03824-3525, USA.
e-mail: andy.rosenberg@unh.edu

1. Watson, R. & Pauly, D. *Nature* **414**, 534–536 (2001).
2. Food & Agriculture Organization of the United Nations *State of World Aquaculture 2006* Fisheries Tech. Pap. 500 (FAO, Rome, 2006).
3. Krkošek, M. *et al. Science* **318**, 1772–1775 (2007).
4. Naylor, R. L. *et al. Issues Ecol.* No. 8 (2001).

NEUROSCIENCE

Love hangover

Leslie C. Griffith

In many species, males have developed strategies to safeguard their genetic material from dilution by that of competing males. Fruitflies achieve this by altering the behaviour of their partners.

Sex can be transformative. Humans often romanticize the after-effects of copulation, but for most organisms there are real biological consequences to mating that go beyond the transfer of sperm. Most species have strategies for protecting their genetic investment that can involve alterations in both the biology and behaviour of the mating partners. For example, in the fruitfly *Drosophila melanogaster* a component of seminal fluid, known as sex peptide, leads to increased egg laying by the mated female and behavioural changes that reduce the likelihood of her re-mating. How sex peptide triggers such a complex array of effects was unknown. On page 33 of this issue, Yapici *et al.*[1] identify the receptor for sex peptide and show that it is expressed in the reproductive tract and in a subset of female neurons believed to be involved in sexual behaviour.

Enhancing the survival of potential progeny is a common goal of males in many species. In mammals, intercourse changes the immunological environment of the female reproductive tract, increasing the probability of successful fertilization and implantation[2]. This type of post-copulatory effect benefits both the male and female partner. For many species there are also other mating associated events that apparently maximize the reproductive success of just one of the involved parties, often at the expense of the other. An obvious example of this is mate guarding. Males of many avian, reptile, rodent, primate and insect species remain close to a recent conquest to lower the probability of her re-mating with a more desirable male and so diluting or displacing their own sperm. The female may benefit in terms of decreased predation, but she loses any opportunity to better the genetic lot of her offspring. The success of this strategy for the male depends on his vigilance, and potentially decreases his chances of mating with other females, so is not without cost.

Nature has also come up with more subtle forms of mate guarding. In snakes[3] and various insect species[4–6], mating can lead to changes

in the female pheromone profile that decrease the attractiveness of mated females to subsequent suitors. Females' behaviour can also be modulated by mating. In rodents, exposure to dominant-male pheromones induces a female preference for dominant versus subordinate males that may involve introduction of new neurons into neural circuits[7]. Similarly, female jewel wasps change their response to male sex pheromones from attraction to aversion after mating[8], and in *D. melanogaster* mated females will actively reject courtship, kicking and running away from a new male[9]. This type of 'mate guarding' does not require the continued presence of the successful male, maximizing his ability to mate with other females while still protecting his DNA investment. Many of these after-effects are generated by chemicals produced by the male and transferred to the female in his ejaculate.

The best-understood example of a behaviourally active seminal-fluid component is sex peptide in *D. melanogaster*[9]. It is produced in the male accessory gland, a prostate-like structure, and is absorbed into the female circulation from the vaginal tract. Behaviours resulting from sex-peptide absorption include increased egg laying and reduced receptivity to male courtship. Previous studies indicated that there are binding sites for sex peptide in the brain[9], but the nature of the receptor or receptors was unknown.

To identify the receptor, Yapici *et al.*[1] carried out an RNA-interference-based genome-wide screen for genes required in females for post-copulatory behaviours; they reasoned that genes whose reduced expression blocked mating-induced increases in egg laying would be candidates. This approach identified a gene for a putative G-protein-coupled receptor. Reducing the expression of this gene did not affect the receptivity of virgin females or sperm storage in mated females, but it completely prevented the development of post-copulatory behaviours in either mated females or virgin females injected with sex peptide.

To determine whether their candidate gene was a bona fide sex-peptide receptor (SPR), the authors expressed it in mammalian cells maintained in culture. They found that sex peptide and DUP99B — a seminal fluid component that can also mediate the switch to mated female behaviour — activate SPR with nanomolar affinity. Other *Drosophila* neuroactive peptides whose receptors are closely related by sequence to SPR did not activate it. SPR homologues from other drosophilids, *Bombyx mori* and *Aedes aegypti*, also responded robustly to sex peptide and DUP99B, indicating that SPR is not unique to *D. melanogaster*.

The distribution of SPR provides some clues to how it might effect behavioural changes. The authors demonstrate that SPR is present both in the reproductive organs of the female and on the plasma membranes of neurons close to the surface of the central nervous system. Previous work by this group[10] has suggested that

MAGEB ROKER/ALAMY

Drosophila neurons that express a putative transcription factor encoded by the *fruitless* gene are required for suppressing mated-female-like rejection behaviours. Yapici *et al.* now find that a subset of these neurons also express SPR, implying that sex peptide modulates these neurons. They used RNA interference to decrease SPR expression in *fruitless*-expressing cells, and overexpressed SPR in mutant flies that could not express it, to demonstrate that expression of SPR in this subset of cells is both necessary and sufficient for the development of post-copulatory behaviour.

For students of neurobehaviour, the most interesting finding of Yapici and colleagues' study is that, in SPR, they now have another specific molecular tag for cells involved in an intriguing and 'plastic' behaviour, allowing them to perform a complete analysis of this behavioural circuit. The more global impact of the work, however, is that it provides a new target for the development of insect-control compounds. That SPR-like receptors exist in most sequenced insect genomes suggests that antagonists of this receptor could be used to reduce insect populations by blocking egg maturation and extrusion, or perhaps SPR agonists could be used to make virgin females reject mating. Although the irony of having their own weapon turned on them will doubtless be lost on the male insects, it is a satisfying and potentially specific approach.

Another question raised by this work is: how widespread is this type of 'mating-induced mind control'? At least one species of vertebrate, the red-sided garter snake, demonstrates mating-induced reductions in female receptivity[3]. Obvious homologues of sex peptide and SPR have not been identified in vertebrates, but this is not surprising given that genes involved in reproduction have a very fast rate of evolution — precisely because they are involved in reproductive behaviour — and this variation promotes the differentiation of species[11]. Although sequence mining has not found much homology along the phylogenetic tree, using more subtle algorithms to analyse protein structure and function indicates that there are fairly striking similarities between components of *Drosophila* and vertebrate seminal fluid[12]. Perhaps there is yet another reason to use a condom, ladies. ■

Leslie C. Griffith is in the Department of Biology, Volen Center for Complex Systems and the National Center for Behavioral Genomics, Brandeis University, 415 South Street, Waltham, Massachusetts 02454-9110, USA.
e-mail: griffith@brandeis.edu

1 Yapici, N., Kim, Y.-J., Ribeiro, C. & Dickson, B. J. *Nature* **451**, 33–37 (2008).
2 Robertson, S. A. *J. Anim. Sci.* **85**, E36–E44 (2007)
3 Mendonca, M. T. & Crews, D. *Horm. Behav.* **40**, 43–50 (2001)
4 Nagaraj shmi, V. K., Applebaum, S. W., Azrie l, A. & Rafael, A. *Arch. Insect Biochem. Physiol.* **64**, 142–155 (2007)
5 E, ma, A. et al. *Curr Biol.* **17**, 599–605 (2007)
6 Kurtovic, A., Widmer, A. & Dickson, B. J. *Nature* **446**, 542–546 (2007)
7 Mak, G. K. et al. *Nature Neurosci.* **10**, 1003–1011 (2007)
8 Ruther, J., Stahl, L. M., Ste ner, S., Garbe, L. A. & Tolasch, T. *J. Exp. Biol.* **210**, 2163–2169 (2007)
9 Kubl i, E. *Cell Mol. Life Sci.* **60**, 1689–1704 (2003)
10 Kvitsiani, D. & Dickson, B. J. *Curr. Biol.* **16**, R355–R356 (2006)
11 Clark, N. L., Aagaard, J. E. & Swanson, W. J. *Reproduction* **131**, 11–22 (2006)
12 Mueller, J. L., Ripol , D. R., Aquadro, C. F. & Wolfner, M. F. *Proc. Natl Acad. Sci. USA* **101**, 13542–13547 (2004)

CARBON CYCLE

Sources, sinks and seasons

John B. Miller

Changes in the phasing of seasonal cycles of carbon dioxide in the atmosphere mark the time when a region becomes a source or a sink of CO_2. One study of such changes prompts thought-provoking conclusions.

We are currently getting a 50% discount on the climatic impact of our fossil-fuel emissions. Since 1957, and the beginning of the Mauna Loa record of atmospheric carbon dioxide, only about half of the CO_2 emissions from fossil fuel combustion have remained in the atmosphere, with the other half being taken up by the land and ocean. In the face of increasing fossil fuel emissions, this remarkably stable 'airborne fraction' has meant that the rate of carbon absorption by the land and ocean has accelerated over time[1]. Unfortunately, we have no guarantee that the 50% discount will continue, and if it disappears we will feel the full climatic brunt of our unrelenting emission of CO_2 from fossil fuels. Indeed, climate models that include descriptions of the carbon cycle predict that terrestrial uptake of carbon will decrease in the next century as climate warms[2]. As they describe elsewhere in this issue (page 49), Piao *et al.*[3] have used observational data to show that rising temperatures may already be decreasing the efficiency of terrestrial carbon uptake in the Northern Hemisphere.

Piao *et al.* looked at changes in the phasing of seasonal cycles of atmospheric CO_2 concentrations at ten sites north of about 20° N. Seasonal cycles of atmospheric CO_2 are caused primarily by the terrestrial biosphere moving from being a net source of carbon to the atmosphere (mainly in winter) to becoming a net sink (mainly in summer), where net carbon uptake or release is determined by the balance between photosynthesis and respiration. Changes in the phasing therefore reflect changes in the timing of when the land is a net sink or source to the atmosphere.

Piao *et al.* used a metric for the phasing known as the 'zero-crossing date' (the ZC date, which is when the seasonal cycle crosses the line that delineates the calculated long-term trend in CO_2 concentration, Fig. 1). They found that higher temperatures led to earlier ZC dates and colder temperatures to later ones. Given the trend towards warmer autumn temperatures, they also found that the ZC was occurring an average of 0.4 days earlier per year. In addition, they identified a temperature correlation with the ZC dates and a trend towards earlier ZC in the spring that was similar to a trend evident in a previous analysis of data from between the 1970s and 1990s[4]. But, most significantly, Piao *et al.* found that the advancement of the autumn ZC was occurring at nearly the same rate as the advancement of the spring ZC, meaning that gains of carbon uptake during spring were being cancelled out by carbon releases in autumn.

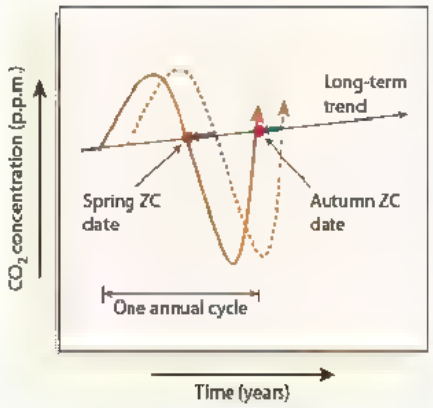


Figure 1 | Zero-crossing (ZC) dates. These dates, shown by red dots, are defined as the time when the annual cycle of atmospheric CO_2 crosses the calculated long-term trend in CO_2 concentration. In spring, this occurs as net CO_2 uptake is increasing and atmospheric CO_2 concentration is falling, and in autumn as net CO_2 release is increasing and atmospheric CO_2 concentration is rising. The phasing of this cycle is determined by net carbon uptake or release throughout the year, which, in turn, is the balance between respiration and photosynthesis. Because net flux is the relatively small difference between the much larger photosynthetic and respiratory fluxes, small fractional changes in either photosynthesis or respiration can have large impacts on the timing of the CO_2 seasonal cycle. Piao *et al.*[3] observed trends (blue arrows, indicating a shift in the cycle from the dashed to solid line earlier each year) in both the spring and autumn ZC date, indicative of a changing balance between photosynthesis and respiration brought on by increasing temperature.

The shrinking autumn uptake signal seems to contradict earlier satellite-derived 'greening' trends[5,6] that showed a lengthening of the growing season in both spring and autumn in the Northern Hemisphere. To better understand this apparent conflict, Piao *et al.*[3] used a computer model of the terrestrial biosphere to help separate the observed 'bottom line' net carbon fluxes of the atmospheric observations into atmospheric debits (photosynthesis) and credits (respiration) that are mechanistically relevant. The model results suggest that increased autumn respiration (triggered by warmer temperatures) dominated over the autumn photosynthetic gains that were seen by the satellites as a longer green period. Moreover, the model also shows that the loss of carbon in autumn seems to largely cancel the uptake gains made by earlier, greener springs, just as the atmospheric data did.

Piao and colleagues' results link temperature and carbon uptake, but using them to predict the future trajectory of carbon uptake is tricky. Even if we know that temperatures will increase, we still need to know temperature trends for spring and autumn. If spring temperatures rise more quickly than those in autumn, sinks could get larger, whereas more rapid increases in autumn temperatures would cause sinks to diminish. Furthermore, the authors point out that, so far, spring temperatures have been rising faster in Eurasia than in North America, whereas autumn temperatures have been rising faster in North America, adding a level of geographical complexity to future projections.

Even for now, however, the picture remains incomplete. Just as measures of greenness from space can't determine total carbon balance because they miss the respiratory side of the equation, so the study by Piao *et al.* doesn't address carbon balance in the winter and summer. And the annual net carbon balance is what is needed in order to understand whether carbon sinks are disappearing, remaining steady or getting stronger.

In light of Piao and colleagues' results, and of two recent studies showing diminishing ocean sinks in the critical carbon-uptake areas of the North Atlantic[7] and Southern Ocean[8], it may seem odd to consider that carbon sinks might be getting stronger. But this is exactly what the steady airborne fraction of global CO_2 is telling us. The global CO_2 signal is most significant for two reasons: first, it is the most robust determination of carbon uptake, because the errors in atmospheric observations and fossil-fuel emissions are very small, and second, the global CO_2 signal is the one that is relevant for the radiative balance that drives global climate change.

So, what gives? For every report of a shrinking sink, there should be even more reports of increasing sinks to satisfy the global constraint. It's possible that we are not looking in all the right places. For example, given the high and increasing[9] amounts of biomass productivity in the tropics, and how poorly observed they are, it would not be surprising if some of the increasing sinks were there. Indeed, some studies show increasing biomass (that is, sinks) in tropical forest plots[10].

Making more observations in the tropics, and in other poorly observed regions in the ocean and on land, will certainly help us find the sinks necessary to balance the global numbers. But as Piao and colleagues' study[3] has shown, to develop greater mechanistic understanding (and thus predictive power), there is also a great need to identify observational constraints on photosynthetic and respiratory fluxes. ■

John B. Miller is at the University of Colorado and the NOAA Earth System Research Laboratory, 325 Broadway, Boulder, Colorado 80305, USA.
e-mail: john.b.miller@noaa.gov

1. Canadell, J. G. *et al. Proc. Natl Acad. Sci. USA* **104,** 18866–18870 (2007)
2. Friedlingstein, P. *et al. J. Clim.* **19,** 3337–3353 (2006)
3. Piao, S. *et al. Nature* **451,** 49–52 (2008)
4. Keeling, C. D., Chin, J. F. S. & Whorf, T. P. *Nature* **382,** 146–149 (1996)
5. Myneni, R. B. *et al. Nature* **386,** 698–702 (1997).
6. Zhou, L. M. *et al. J. Geophys. Res.* **106,** 20069–20083 (2001)
7. Schuster, U. & Watson, A. J. *J. Geophys. Res.* **112,** doi:10.1029/2006JC003941 (2007)
8. Le Quéré, C. *et al. Science* **316,** 1735–1738 (2007)
9. Nemani, R. R. *et al. Science* **300,** 1560–1563 (2003).
10. Baker, T. R. *et al. Phil. Trans. R. Soc. Lond. B* **359,** 353–365 (2004).

OPTICS

Watch your back

Kosmas L. Tsakmakidis and Ortwin Hess

A proposal for transporting photons invisibly between two unconnected points in space seems worthy of a *Star Trek* plot. But it is in principle wholly realizable, and could open up new vistas — literally.

Imagine walking down a footpath, staring unconcernedly at the clear track in front of you. Suddenly, you stumble over an object. You look down, but there is nothing to be seen on the ground. You step back and try a different angle of view, again without success. But you know something must be there, because you can feel it.

This situation is brought a step closer to reality with a device dreamt up by Greenleaf *et al.*[1] and described in *Physical Review Letters*. The authors propose a way of creating an 'invisible tunnel' through which photons, the elementary particles of light, can propagate between two seemingly unconnected points. To an observer standing where they emerge from their tunnel, the photons seem to come from nowhere. To an external viewer, they seem to be teleported from one place to the other. And anything within the tunnel cannot be seen by anyone. In analogy to the infamous 'wormholes' — a prediction in general relativity of tunnels through space-time that connect distant areas of the Universe — the authors call their brainchild an electromagnetic wormhole.

The wormhole works (in theory) by neatly combining concepts from differential geometry, general relativity, electromagnetism and the theory of 'metamaterials'[2]. Metamaterials are composite, nanostructured materials with specifically tuned electromagnetic properties. The authors construct the tunnel wall of their wormhole using a metamaterial layer that is designed to bend light waves around it without reflection, much as water waves bend around a tree branch or similar obstruction lying just below the surface of the water. This layer thus renders whatever is inside it invisible. The idea draws on techniques proposed[3,4] for the creation of an 'invisibility cloak' (Fig. 1a) — a device that has already been constructed and proved viable, at both microwave[5] and optical[6] wavelengths.

The advance in Greenleaf and colleagues' scheme[1] is that a cloaked object can 'see' into the outside world at the end of the tunnel, because photons are also free to propagate through it. The tunnel 'deceives' photons into thinking that remote regions are connected to each other so that they naturally follow the path inside the cylindrical channel. This cylinder is not part of conventional three-dimensional space, but is part of a higher-dimensional space outside it. Its topology is rather like the handle of a coffee cup connecting two areas of the cup's surface. If the handle is hollow and has two open ends, it presents an alternative route (other than staying on the surface of the cup) for getting from the one place to the other.

The key to the realization of this scheme is that this new photon-space does not naturally exist in real space. Rather, using suitable coordinate transformations, the authors tweak Maxwell's equations — the set of equations that describe the workings of electromagnetic waves — to simulate it. The equations retain their form on passing from the real to an artificial photon-space; the only thing that is required to complete the deception of the photons is to modify the values of the electric permittivity and magnetic permeability (numbers that codify the degree to which a material allows electric and magnetic fields to pass).

Such a concealed communication channel could be deployed for military purposes for the secret transmission of information or stealth technologies. But it might also find its way into civilian applications: rerouting mobile-phone signals around obstacles, for example, or shielding sensitive medical devices from interference by magnetic resonance imaging scanners. But the possibilities don't end there. Two of Greenleaf and colleagues' invisible electromagnetic tunnels, built into the frame of a pair of special half moon spectacles, would effectively 'glue' the photon-space behind the head to the photon-space in front of the eyes, allowing one literally to watch one's back (Fig. 1b).

Currently, metamaterial technology allows the construction of invisibility cloaks that work well for only a limited range of frequencies. The electromagnetic wormhole in its present form can also be only short, otherwise the image of an object being transmitted through it becomes noticeably distorted. The true potential of such schemes will become clear in future experimental tests. What is plain now is that innovations are coming thick and fast in this burgeoning world of 'transformation optics'. ■

Kosmas L. Tsakmakidis and Ortwin Hess are at the Advanced Technology Institute and the Department of Physics, University of Surrey, Guildford GU2 7XH, UK.
e-mail: o.hess@surrey.ac.uk

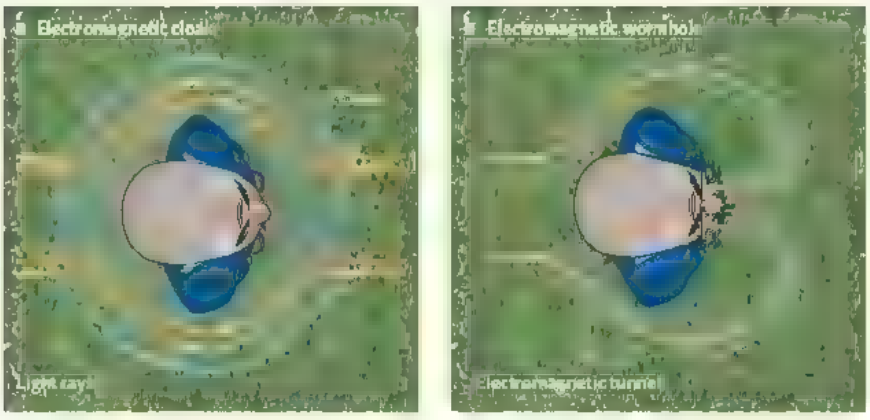


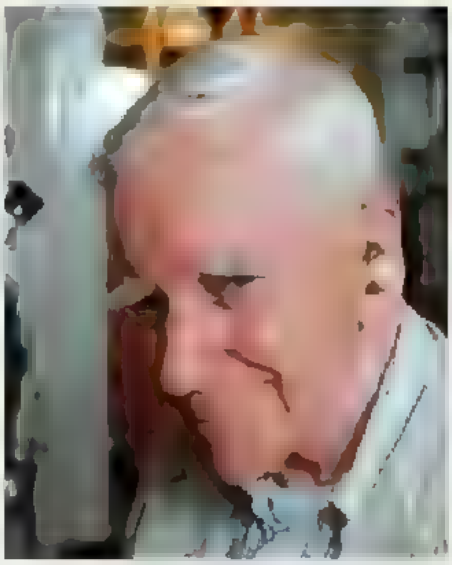
Figure 1 | It's behind you. a, The invisibility cloak devised by Pendry *et al.*[3] uses specially structured 'metamaterials' to open up a 'hole' in photon space, inside which one can place an object. Photons are naturally redirected around the object, rendering it invisible, at least when viewed with photons at a certain wavelength. **b**, Greenleaf and colleagues' electromagnetic wormhole[1] is a natural extension of the invisibility idea, with exciting potential applications. For example, in principle two flexible wormholes attached to the frame of a specially designed pair of half-moon spectacles could project the photon space behind the head to the half moon area of the lenses, providing a seamless 360° view.

1. Greenleaf, A., Kurylev, Y., Lassas, M. & Uhlmann, G. *Phys. Rev. Lett.* **99,** 183901 (2007)
2. Eleftheriades, G. V. & Balmain, K. G. *Negative Refraction Metamaterials. Fundamental Principles and Applications* (Wiley-IEEE Press, Hoboken, NJ, 2005)
3. Pendry, J. B., Schurig, D. & Smith, D. R. *Science* **312,** 1780–1782 (2006)
4. Leonhardt, U. *Science* **312,** 1777–1780 (2006)
5. Schurig, D. *et al. Science* **314,** 1780–1782 (2006)
6. Cai, W., Chettiar, U. K., Kildishev, A. V. & Shalaev, V. M. *Nature Photon.* **1,** 224–227 (2007)

OBITUARY

Alan J. Southward (1928–2007)

Marine biologist: a pioneer of marine ecosystem time series.



G. BRAASCH

Alan Southward's many contributions set him among the most influential figures of twentieth-century marine science. Much of his career was spent at Plymouth, in Devon, an ideal location for studying how environmental changes affect marine ecosystems: the western English Channel and its approaches form a boundary between oceanic and coastal waters, as well as between two biogeographical provinces.

In the 1890s, plankton surveys were started in this region by the Marine Biological Association of the United Kingdom (MBA). Several time series were established, including those of zooplankton by Frederick Russell in the 1930s. Southward started baseline surveys of intertidal organisms around the British and Irish coasts in the 1950s, and later took over the Plymouth zooplankton time series. Using old records, he demonstrated how the distribution of organisms changed with changing sea temperatures. This was long before the advent of concern about global warming; his work laid the foundations for subsequent studies of climatic effects on marine ecosystems.

Southward was born in Liverpool, the son of a Cunard engineering fitter. He contracted meningitis at 15, with the result that he lost his hearing and sense of balance, and had to learn to balance by eye. An interest in shore organisms led him to study zoology at the University of Liverpool, graduating in 1948. Doctoral studies followed, on the ecology of intertidal animals, at Port Erin Marine Laboratory on the Isle of Man. It was there that he met, and later married, his lifetime scientific partner, Eve Judges.

In 1953, the year the MBA acquired the side-trawler *Sarsia* for offshore studies, Southward moved to Plymouth as a postdoctoral fellow. He was on the MBA staff from 1956 until his retirement in 1988, and continued working there until his death on 27 October 2007. He initially followed in the steps of Charles Darwin, the most famous student of barnacles, in showing how studies on barnacle distribution could allow inferences to be made about biogeographical barriers. His early intertidal surveys were used to quantify the damage caused by the detergent used to disperse the *Torrey Canyon* oil spill in 1967; more notably, he showed how long it took for the ecosystem to recover.

From the early 1950s onwards, Southward showed how minor changes in environmental conditions, especially temperature, correlated with changing geographical ranges of intertidal and planktonic organisms. He worked with Russell, and others, describing the events leading to the 1930s collapse of the herring fishery and the replacement of herring by pilchards. Their seminal paper, published in 1971, linked changes in water mass and water chemistry to changes in plankton species and abundance. Subsequent studies on cyclical and long-term changes in the marine ecosystem led to an understanding of the causes of these changes, and to predictions of the consequences of rising sea temperatures.

The Southwards first used *Sarsia* for deep-sea studies in 1956, dredging the continental slope for barnacles, which involved long hours of noisy winch work. The scientists' cabins were directly under the winch, making sleep difficult — except for Southward, whose deafness proved a boon (likewise his immunity to seasickness, although he was, once, reported to have felt uncomfortable in a force 12).

Looking through dredge sievings, the Southwards noticed hair like organisms that had probably been ignored previously because they resembled the fibres of the dredge nets. They became fascinated by these small, mouthless, gutless tubeworms, known as pogonophores, and investigated whether they could obtain nutrition from dissolved organic compounds in the sediment. Following the 1977 discovery of hydrothermal vents and giant pogonophores (vestimentifera) on the Galapagos Rift, Colleen Cavanaugh showed that sulphur-oxidizing bacteria living symbiotically within the vestimentiferans supplied them with their nutrition by chemosynthesis. It soon emerged that the small pogonophores of the continental slope also contained such bacteria.

Several cruises to the slope were spent looking for the bacterial energy source because, unlike the vents, hydrogen sulphide and methane were almost absent there. Finally, Southward negotiated funding to study a shallow pogonophore habitat near Bergen, Norway. Two bivalve species were found, also with chemosynthetic bacteria, living alongside the pogonophores. Eventually, it was deduced that all these organisms 'mined' sedimentary iron sulphides to obtain energy for bacterial carbon dioxide fixation. In this way, chemosynthetic ecosystems were shown to be widespread in reducing sediments, from the intertidal regions to the deep sea.

Southward continued studying pogonophores throughout his life, a final paper being completed just before his death. Research came full circle with the conclusion that many pogonophores are 'mixotrophic', depending on both endosymbiotic bacteria and dissolved organic matter.

In retirement, Southward turned to the study of hydrothermal vent ecosystems. This was aided by an invitation to become an adjunct professor at the University of Victoria, Canada, to study vents in the Pacific. He was an early user of stable-isotope data to follow nutritional pathways in such ecosystems in the Atlantic, Mediterranean and the Pacific.

Southward was an excellent writer and stimulating speaker. For 20 years he was a meticulous editor of *Advances in Marine Biology*, with an uncanny eye even for the missing comma in a reference list. He encouraged scientists from the Soviet Union to publish reviews in English to gain a wider audience. This led to a heavy editing workload, not least in coping with the authors' 'Russish', but Southward justifiably obtained great satisfaction from seeing their work in print. Following the break-up of the Soviet Union, there was little funding for Russian marine biologists, and Southward worked hard to find Western sources of money to keep them in post.

Alan Southward was an innovative, multi-disciplinary scientist, one measure of his encyclopaedic knowledge being the impression he left on postgraduates at Victoria by his ability to advise them on any research project. He published prolifically, with 11 papers stemming from his doctoral studies alone; overall, he published 200 papers, along with two books (*Life on the Seashore* and *British Barnacles*). He could be impatient, especially with administrators who took a short term view, but devoted much time to encouraging young scientists. Southward was one of the greatest marine biologists of his generation, and leaves an internationally regarded legacy of dedication and achievement.

Paul R. Dando

Paul R. Dando, of the Marine Biological Association of the United Kingdom, is at the School of Ocean Sciences, Bangor University, Menai Bridge LL59 5AB, UK.
e-mail: oss109@bangor.ac.uk

Q&A

ESA/C. CARREAU

ASTRONOMY

Extrasolar planets

Dimitar D. Sasselov

Hundreds of planets are known to orbit stars other than the Sun, and unprecedented observations of their atmospheres and structures are being made. It's an invaluable opening for understanding the planets' diverse natures, the formation of our Solar System, and the possibility of habitable planets beyond our home.

How many planets outside our Solar System have we found so far?
The number is going up week by week, but at the moment there are about 270 confirmed extrasolar planets — exoplanets, as they're known in the jargon. Not bad, considering the first one was discovered just 13 years ago, and they're not that easy to spot.

What makes exoplanets difficult to find?
They are, by definition, far away and orbiting close to a star that is far bigger and brighter than them. Light contrast ratios of 10^{10} (at visible wavelengths) to 10^{7} (in the infrared) between a star and planet make direct detection by imaging extremely hard. Exploiting mass ratios, which are generally in the region of 10^{3}–10^{5}, is slightly less daunting. Indeed, the most popular way of spotting an exoplanet is through a star's 'wobble', which is caused by the gravitational pull of an orbiting planet. This is measured through the Doppler effect — a shift in wavelength — in the spectrum of visible light from the star. It's a tiny effect, proportional to the mass ratio. Measuring it requires patience, because the wobble must be followed for at least one complete orbit of the planet. A wobble can also be detected directly by carefully observing the position of the star with respect to other stars (a technique known as astrometry), but this is technically even more demanding.

What other ways are there of finding exoplanets, besides the Doppler effect?
Gravitational lensing is another common method, and also exploits the star–planet mass ratio. For this, a source of light is needed, usually another star, behind the star being investigated. As we look at it, the light from the background star will be bent by the gravity of the intervening star. If the intervening star has an attendant planet, this will alter the lensing effect in a noticeable way.

And what about the transiting method?
Transiting is perhaps the most fruitful method of observation, because of the information we can glean about the planet. It exploits the least daunting inequality between star and planet — the factor 10 to 100 difference in size. As it passes across the disk of its parent star, a planet will dim the star's light by a fraction $(R_p/R_*)^2$, where R_p and R_* are the respective radii of the planet and star. For a planet the size of Jupiter and a star the size of the Sun, this dimming effect would be roughly 1%, which is easily detectable even with amateur equipment. The catch is that a transit requires the planet's orbit to be almost exactly edge-on as we look at the star, which is extremely unlikely. Tens of thousands of stars must be monitored with patience to detect periodic dimming in just a handful.

What can these crude remote-sensing techniques tell us about exoplanets?
A surprising amount. The Doppler technique gives the size, period and eccentricity (deviation from a perfect circle) of the planet's orbit, as well as a quantity $M_p \sin(i)$ that contains both the planet's mass M_p and the inclination, i, of its orbit to our line of sight. The transit technique contributes the planet's radius and a direct value for i. Combining these two parameters can thus provide the actual value of M_p, and also — a big prize, this, because it tells us something about what the planet is made of — the planet's mean density, calculated from

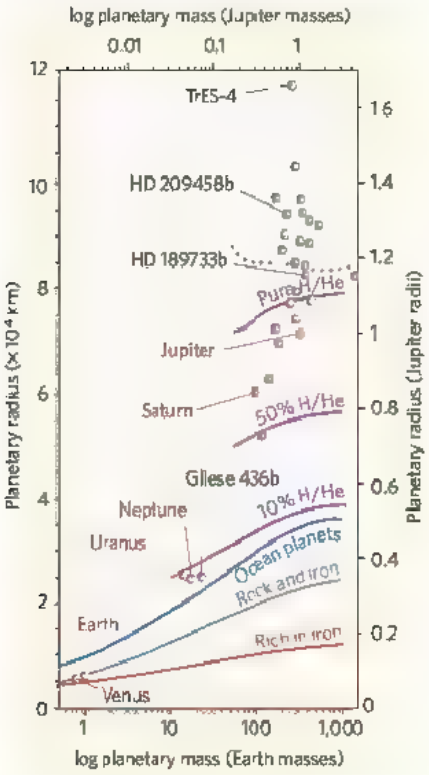


Figure 1 | The gamut of transiting exoplanets. The composition of a planet will determine its average density and where it will lie on a plot of radius against mass. Here, various planets within (red circles) and beyond (grey circles) the Solar System are compared with models of different interior composition: from an interior made of rock and iron alone (Venus and Earth approximate to this state) to a gaseous composition of just hydrogen and helium (of which Jupiter is the closest example in our Solar System). Owing to difficulties in spotting such small bodies, few exoplanets at the small, dense end of the scale have so far been found. Many giant planets have been detected that have very low densities above the upper range of pure hydrogen–helium planets (dotted line) — the exoplanet TrES-4 is the most extreme example — and these are a serious challenge to theory.

the radius and the mass. More than 20 transiting planets are already known, and the radius and mass of several have been determined to better than 4% and 8% accuracy, respectively. But there's a sting in the tail of this simple, surprisingly accurate method — most of the planets found so far are much less dense than our theories predict (Fig. 1).

Why are less-dense planets a problem for theoretical models?

Most exoplanets known so far are Jupiter-like 'gas giants', with masses between 0.5 and 3 times that of Jupiter (150–1,000 Earth masses). But they have an unexpectedly wide range of sizes, with radii between 0.8 and 1.7 times Jupiter's. Jupiter is thought to have a small core of heavy elements, surrounded by envelopes of hydrogen with some helium intermingled. Explaining an exoplanet with a similar mass to Jupiter but a smaller size (higher mean density) is easy — it probably has a larger core, with more heavy elements in general. But for exoplanets that are less dense, there comes a point when even a planet made just of hydrogen isn't enough to explain its low density.

How can we get around this difficulty?

A possible explanation is additional heating, either because of some persistent heat source or a seriously delayed cooling of the planet. One obvious source of heat is the parent star itself: the exoplanets in Figure 1 are mainly 'hot Jupiters' that orbit extremely close to their stars. But then it's unclear why some planets would absorb much of that heat and others would not. The same problem — accounting for the entire range of observed planetary sizes — also bedevils other proposed solutions.

What more can remote sensing tell us about a planet, besides basic features such as its size and density?

This is where transiting exoplanets come into their own. Their well-constrained basic parameters and natural 'on–off switch' allow their weak signal to be calibrated accurately. That means we can perform basic spectroscopy: atomic sodium and hydrogen have already been detected in exoplanets' upper atmospheres. Unprecedented spectroscopy and thermal mapping of the atmosphere of the transiting hot Jupiter HD 189733 b has been possible at the point where it is seen 'side-on', just as it disappears behind its star. Here, the infrared light flux from the planet can be discerned at a level just 10^{-4} that of the star's flux. NASA's infrared Spitzer space telescope has proved perfect for this task.

What have we learnt about the atmosphere of HD 189733 b?

First, we have a measure of the temperature difference between the day and night side, which is about 230 °C. In such an extremely close orbit — HD 189733 b circulates around its star at just 3% of the Earth–Sun distance — a planet's rotation and orbital revolution become synchronized, with one side permanently facing the star. (In the same way, the Moon always presents the same side to Earth.) During transit, we thus see the night side, and the heat flux we see coming from this side indicates that the planet's atmosphere must transport heat very efficiently from the day side. The hottest and coldest regions are not, as would be expected, seen at the longitudes facing exactly towards and away from the star, respectively, but are slightly offset from these longitudes, indicating a very dynamic, windy atmosphere.

Is there evidence for water on any exoplanet?

It would be surprising not to find some water in the atmospheres of hot Jupiters: water is very common in the low-temperature environments where planets form and evolve, owing to the large cosmic abundance of both hydrogen and oxygen. Absence of water might indicate thermal and radiative loss, as seems to have been the case with our small and hot planetary neighbour Venus. Hot Jupiters should be massive enough to stave off complete loss, but then again we know little about the extreme conditions in their upper atmospheres. By measuring the 'depth' (the loss or gain in intensity) of eclipse or transit in different infrared wavelength bands, Spitzer can crudely sample the molecular lines not only of water, but also of other gases of interest, such as carbon dioxide and methane. Water has already been seen on three hot Jupiters — TrES-1, HD 209458 b and HD 189733 b.

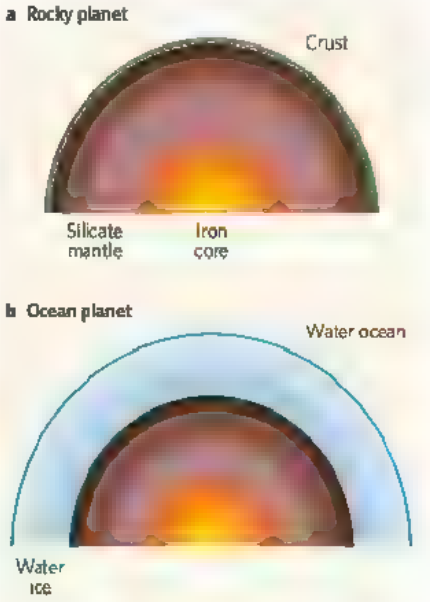


Figure 2 | Journey to the centre of a super-Earth. Super Earth planets, with masses between two and ten times that of Earth, come in at least two varieties: **a**, rocky; **b**, ocean. Both types originate in a well-mixed structure of solids (silicates) and volatiles (such as water and ammonia), with trace amounts of hydrogen and noble gases. The structure differentiates very quickly and in a strictly predictable way: iron and siderophile elements (high density transition metals that like to bond with iron) precipitate into a core, and excess water, ammonia and so on precipitate above a silicate mantle. If water is present in significant quantities, most of it will be under pressures in excess of 10 gigapascals, and thus in a solid state — ice — despite the high temperatures. An ocean planet will be larger than a rocky one owing to the lower density of water, and the size of a planet of a given mass will depend on the proportion of core, mantle and water. Models show that an uncertainty of less than 5% in radius and less than 10% in mass are needed to distinguish ocean from rocky planets.

We seem to know of many larger, Jupiter-sized planets — what about Earth-like planets?

First off, the rich diversity of giant exoplanets came as a surprise to astronomers — we expected to find a greater variety of 'terrestrial'

planets. But to date we know of only a handful. None of them is transiting, so we don't know their mean densities or even their exact masses. And 'terrestrial' is perhaps a misnomer: the masses of such planets discovered so far are between 5 and 10 times Earth's mass (M_E). Such 'super-Earths' do not feature in our Solar System, but are seemingly common elsewhere. The limit of 10 M_E is theoretically inspired: when planets form in a typical protoplanetary disk of gas and dust, 10 M_E is roughly the critical mass above which hydrogen can be accreted and retained by the growing planet, turning it into a Neptune-like giant. Given the diversity of models for predicting the composition of the planet interiors at 10 M_E, we need a large sample of planets and their mean densities to check this.

	Mass (Earth masses)	Distance from star (AU)	Density (Earth densities)	Mean surface temperature (K)	Notable features
Venus	0.8	0.7	0.95	735	Sweltering CO_2 atmosphere
Earth	1	1	1	287	Life
Gliese 581c	>5	0.07	>0.7	>400	Hot super-Earth
Gliese 581d	>8	0.25	>0.8	<280	Cool super-Earth — habitable?
Neptune	17	30	0.30	72	Vivid blue colour — cold
Gliese 436 b	23	0.03	0.32	712	Neptune-like, but orbiting close around a red dwarf
HD 209458 b	219	0.05	0.07	1,600	Hydrogen, sodium and possibly water vapour
TrES-4	267	0.05	0.04	2,100	Largest known planet in size — but would float on water
Jupiter	318	5.2	0.24	165	The Great Red Spot — a violent, centuries-old storm
HD 189733 b	366	0.03	0.18	1,200	Efficient heat transfer in a turbulent atmosphere
XO-3 b	~4,400	0.05	0.4–2.0	Unknown	Most massive planet known — a planet, or a failed star?

Figure 3 | A world of worlds. Our own Solar System is home to an amazing variety of planets (examples in red), from the small, rocky sisters Earth and Venus to the gas giants Neptune and, largest of all, Jupiter. But that is nothing compared with the diversity that is emerging among exoplanets, as this selection of particularly notable examples shows. Limitations on current observational capabilities favour the discovery of exoplanets that are particularly large (and are therefore gas giants), and those orbiting very close to their stars. 1 astronomical unit, AU, is the Earth–Sun distance; data for exoplanets can by their nature be highly uncertain.

Why do astronomers expect a great diversity among smaller planets?

Because smaller, solid planets can have more varied material compositions. Depending on how its material compresses, cools and mixes, and what it weighs, a planet's interior can come to be dominated by an iron or iron–alloy core, a silicate mantle, (mostly water) ice, or a hydrogen–helium envelope. A rare fifth type of interior material is a carbon-rich mantle. In general, we expect two distinct families: 'ocean planets', with water making up more than 10% of their mass; and rocky, Earth-like planets (water makes up only about 0.05% of Earth by mass) that may still have oceans (Fig. 2).

So should we expect other solar systems just as diverse as our own?

Yes indeed — there's no reason to think that our Solar System is special in any way; and equally, we are beginning to realize that there is a great diversity of planetary types not represented in our own backyard (Fig. 3). Since early 2007, for example, we have spotted at least three planets orbiting the star Gliese 581. Two of them are super-Earths, but they do not transit, so we know only their minimum possible mass through the term $M_p\sin(i)$ — 5 and 8 M_E — and their orbital distances, which are 7% and 25% of the distance from Earth to the Sun, respectively. Because Gliese 581 is small, cool and faint (it is 75 times less luminous than our Sun), the tiny orbits of these planets mean that their surface temperatures might range from that of Venus (around 460 °C) down to that of Mars (a mean temperature of −55 °C). One of the planets, Gliese 581 d, might have a temperature similar to that of Earth's polar regions.

Could any of these planets be habitable?

Probably not: the inner, 5-M_E super-Earth is probably too hot, and the outer one is probably too cold, although still potentially habitable. But because they do not transit, we have no constraints on their bulk composition — except that the existence of a third, Neptune-mass planet implies the presence of gravitational forces that caused them to migrate from an initial location farther out in the protoplanetary disk. The two super-Earths are thus probably ocean planets, as they must have formed outside the disk's snow line, where water freezes and accretes easily; some of this surface water would have liquefied when the planets were pushed closer in.

What will be the next stage of exoplanet discovery?

It will be to match recent observational breakthroughs with breakthroughs in our understanding. For that, we need a large enough sample to encompass the full diversity of planets out there — meaning many more hundreds, if not thousands. That will be achieved quickly in the coming years with results from dedicated space missions such as the French-led COROT (for 'COnvection, ROtation and planetary Transits'), which was launched in December 2006, and NASA's Kepler, scheduled to launch in early 2009. In the meantime, with advances in remote-sensing techniques, exoplanet science is already shifting from mere discovery to exploration. As planet hunters hone their tools to discover smaller and smaller planets, more surprises should be in store.

When will we find another Earth?

The goal of characterizing Earth-like planets — even true analogues of Earth — is very exciting, and is feasible before 2020. By that time, spectroscopy at very low resolutions could allow us to see signatures of familiar global planetary cycles (the carbonate–silicate cycle, which stabilizes Earth's climate over long timescales, for example), and perhaps let us take the first steps in the search for markers of biological entities. The essential precondition is that we know something of the make-up of the planet in question — that is, its mass and radius. Kepler is designed to find Earth-sized planets by 2014 using transits. Following this up with spectroscopy will sort out the planets' true nature.

And would another Earth necessarily be habitable?

The first terrestrial planets that we will be able to study in detail will be super-Earths that transit small stars. We are likely to learn a lot of geophysics from them, and, who knows, they might turn out to have excellent habitable potential as well. But ultimately, we'll have to anchor our theoretical models to the only true reference — Earth. Exploring planets down to Earth's size remains a priority of enormous resonance, both scientific and emotional. ■

Dimitar D. Sasselov is in the Department of Astronomy, Harvard University, Cambridge, Massachusetts 02138, USA, and the Harvard Origins of Life Initiative.
e-mail: dsasselov@cfa.harvard.edu

FURTHER READING
Burrows, A. *Nature* **433**, 261–268 (2005).
Knutson, H. A. *et al. Nature* **447**, 183–186 (2007).
Selsis, F. *et al. Astron. Astrophys.* **476**, 1373–1387 (2007).
Tinetti, G. *et al. Nature* **448**, 169–171 (2007).
Udry, S. *et al. Astron. Astrophys.* **469**, L43–L47 (2007).
Valencia, D., Sasselov, D. D. & O'Connell, R. J. *Astrophys. J.* **665**, 1413–1420 (2007).

nature REPORTS climate change

The Climate Change Podcast

Tune in over the next three months for interviews with the people behind the science, insights from journalists covering the research, and opinions from climate experts.

Each of the three monthly shows will report on 'ssues before, during, and after the UN Cl mate Conference in Bali.

In the first instalment, discover elements that could tip us over into dangerous climate change, look at the future for the IPCC, and learn how the ´escalator effect' is killing off species.

Free to download from the *Nature Reports Climate Change* website
www.nature.com/climate

Produced with support from NERC

NATURAL ENVIRONMENT RESEARCH COUNCIL

nature publishing group npg

ARTICLES

A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour

Nilay Yapici[1]*, Young-Joon Kim[1]*, Carlos Ribeiro[1] & Barry J. Dickson[1]

Mating in many species induces a dramatic switch in female reproductive behaviour. In most insects, this switch is triggered by factors present in the male's seminal fluid. How these factors exert such profound effects in females is unknown. Here we identify a receptor for the *Drosophila melanogaster* sex peptide (SP, also known as Acp70A), the primary trigger of post-mating responses in this species. Females that lack the sex peptide receptor (SPR, also known as CG16752), either entirely or only in the nervous system, fail to respond to SP and continue to show virgin behaviours even after mating. SPR is expressed in the female's reproductive tract and central nervous system. The behavioural functions of SPR map to the subset of neurons that also express the *fruitless* gene, a key determinant of sex-specific reproductive behaviour. SPR is highly conserved across insects, opening up the prospect of new strategies to control the reproductive and host-seeking behaviours of agricultural pests and human disease vectors.

At various stages in their lifespan, animals can undergo marked switches in their innate behavioural patterns. Such behavioural switches are attractive models to explore the genetic and neural control of innate behaviours more generally, and are particularly apparent in the dimorphic behaviours involved in mating and reproduction. For example, males and females of most species have distinct mating behaviours that are usually specified during development[1,2], but in some species these can also be switched in the adult[3]. In *Drosophila melanogaster*, the switch that specifies male or female mating behaviour is thought to be set during development[1] by the sex-specific transcripts of the *fruitless* (*fru*) gene[4,5].

Another type of behavioural switch found in many species is the marked change in female behaviour that occurs on mating. For example, in many insect species, virgin females are receptive to courting males and retain their eggs, whereas those that have recently mated are unreceptive and lay eggs. These changes in female behaviour are induced by factors present in the male seminal fluid[6]. In *Drosophila*, the primary trigger of this behavioural switch is the sex peptide (SP), a 36-amino-acid peptide produced in the male accessory gland[7–9]. How SP exerts its effects on female behaviour is unknown, although it has been suggested that the SP might act in part by modulating the activity of neurons that express *fru* (ref. 10). Here, we identify a SP receptor, SPR, and show that it is specifically required in the *fru* neurons for the post-mating switch in female reproductive behaviour.

SPR mediates the post-mating switch

We identified the gene *CG16752*, henceforth referred to as *SPR*, in a genome-wide transgenic RNA interference (RNAi) screen for genes required in the female nervous system for post-mating reproductive behaviour. Specifically, we found that pan-neuronal expression of an RNAi transgene[11] targeting *SPR* (*elav-GAL4 UAS-SPR-IR1*) led to a marked reduction in egg laying. To examine this egg-laying phenotype more carefully, and to assess other reproductive behaviours, we used a protocol in which individual virgin females were first tested for receptivity towards a naive male (Fig. 1a). Those females that mated were then allowed to lay eggs for 48 h before being retested for receptivity with a second naive male. In the initial mating assays, virgin *SPR* RNAi females were as receptive as the control females (Fig. 1b). However, in contrast to control females, *SPR* RNAi females laid very few eggs after mating (Fig. 1c), mated again at high frequency (Fig. 1d), and did not actively reject the second male (Fig. 1e). In all these assays, mated *SPR* RNAi females behaved indistinguishably from wild-type virgin females, as well as from females previously mated to *SP* null males (Fig. 1c–e).

To control for potential off-targeting effects of the initial RNAi transgene, we generated a second independent line, *UAS-SPR-IR2*, which targets a different region of the *SPR* gene (Fig. 1f). In all four assays, this new RNAi line gave results similar to those obtained with the original line from the genome-wide library (Fig. 1b–e). We also identified a molecularly defined deficiency[12], *Df(1)Exel6234*, which removes 88 kilobases (kb) from the X-chromosomal region that includes *SPR* and four other annotated genes (Fig. 1f). We verified the molecular breakpoints of this deficiency and confirmed that it deletes the entire *SPR* gene. Females homozygous for this deficiency were fully viable and had no obvious defects in the gross anatomy of their nervous system or reproductive organs. When tested in parallel in the same series of receptivity and egg-laying assays, *Df(1)Exel6234* homozygous females showed the same post-mating defects as observed on RNAi knockdown of *SPR* (Fig. 1b–e).

By mating *SPR* RNAi or deficiency females to males with sperm labelled by GFP (green fluorescent protein)[13], we confirmed that sperm were transferred and stored normally in these animals. The few eggs laid by these females are also fertilized and develop normally. We therefore postulated that the abnormal post-mating behaviours of these females could be due to a lack of sensitivity to SP, rather than due to a more general defect in reproductive physiology. To test this, we injected SP into the haemolymph of *SPR* RNAi or deficiency virgin females. The receptivity of these females was then tested 5 h later in pairings with naive wild-type males. As controls, we confirmed[14] that wild-type virgins injected with SP were unreceptive, whereas those injected with buffer alone were just as receptive as uninjected virgins (Fig. 1g). In contrast, *SPR* RNAi and deficiency virgins remained receptive even after injection with SP (Fig. 1g).

[1]Research Institute of Molecular Pathology (IMP), Dr. Bohr-Gasse 7, A-1030 Vienna, Austria
*These authors contributed equally to this work

These genetic data demonstrate that *SPR* is required in the nervous system for the behavioural switch triggered by SP.

SPR is a specific SP receptor

The *SPR* gene is predicted to encode a G-protein-coupled receptor (GPCR). To test whether this GPCR might be the SP receptor itself, we expressed *SPR* complementary DNA in mammalian Chinese hamster ovary (CHO) cells together with the Ca^{2+} reporter aequorin. In this assay, ligand-mediated GPCR activation triggers a luminescent flash by means of the $G\alpha_q$- or $G\alpha_{16}$-dependent Ca^{2+} pathway[15]. We detected only a very weak response to SP in these cells, even at concentrations as high as 10 μM (Fig. 2a). It has been suggested that SP responses might involve the cAMP rather than the Ca^{2+} pathway[16,17], and so one reason for this poor response might be that SPR normally couples to G proteins other than $G\alpha_{q/11}$. Accordingly, we cotransfected these cells with constructs encoding one of three different chimaeric G proteins ($G\alpha_{qs}$, $G\alpha_{qi}$ or $G\alpha_{qo}$) designed to divert $G\alpha_s$-, $G\alpha_i$- or $G\alpha_o$-dependent signals, respectively, from the cAMP pathway into the Ca^{2+} pathway[18,19]. Indeed, co-expression of $G\alpha_{qi}$ or $G\alpha_{qo}$, but not $G\alpha_{qs}$, resulted in robust Ca^{2+} responses to SP (Fig. 2a).

The response to SP is highly specific, because we did not detect comparable levels of activation by any of eight other *Drosophila* peptides, even at 10 μM (Fig. 2b; see Methods). Amongst the closest relatives of SPR in *Drosophila* are CG2114 (also known as FR) and CG8784, receptors for FMRFamides and hugin-γ, respectively[20–23]. Neither FMRFamide nor hugin-γ activated SPR, and, conversely, expression of CG2114 or CG8784 in CHO cells conferred sensitivity to their respective ligands, but not to SP (Fig. 2b). In a dose–response assay, we determined that SP activates SPR with an effector concentration for half-maximum response (EC_{50}) of 1.3 nM (Fig. 2c). The closely related peptide, DUP99B, which induces the same post-mating responses as SP in injection assays[24], activates SPR with an EC_{50} of 7.3 nM. Thus, both SP and DUP99B specifically activate SPR at physiological concentrations, with EC_{50} values in the low nanomolar range typical for such peptide–GPCR interactions[22]. We conclude that *SPR* encodes a functional receptor for SP that couples to $G\alpha_i$ and/or $G\alpha_o$ to regulate cAMP levels.

SPR is in the reproductive organs and the CNS

To define the cellular targets of SP, we generated antisera against an amino-terminal region of SPR. These antisera revealed high levels of SPR expression in the female reproductive organs, in particular in the spermathecae, the primary sites for long-term sperm storage[25], and the lower oviduct (Fig. 3a, c, d). Staining with the anti-SPR antisera was restricted to the cell membrane (Fig. 3d) and was absent in *Df(1)Exel6234* homozygous females (Fig. 3b), confirming the specificity of our antisera. SPR could not be detected in the male reproductive organs.

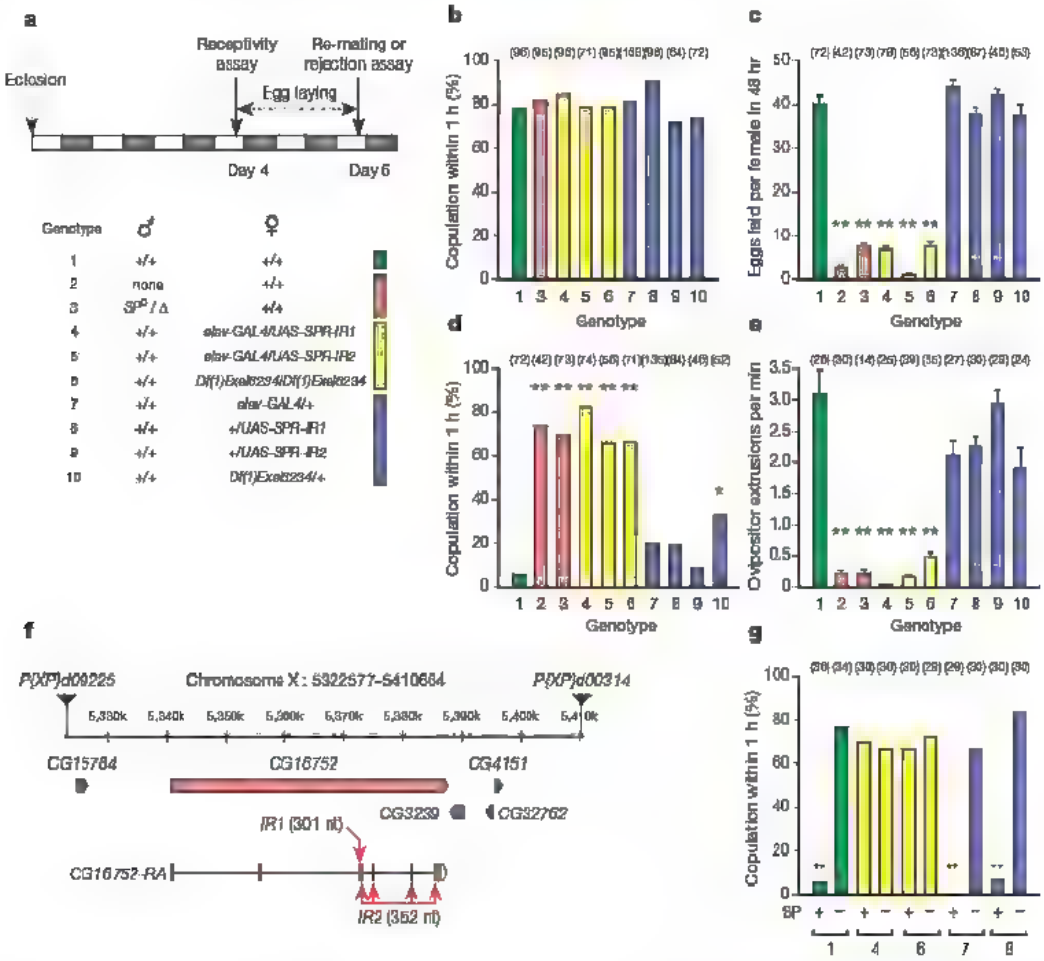


Figure 1 | *SPR* is required for the post-mating switch induced by SP. **a**, Protocol for behavioural experiments in **b**–**e**. **b**, Receptivity of virgin females, scored as the percentage of females that copulated within 1 h. $P > 0.01$ for all comparisons against +/+ (genotype 1); χ^2-test with Bonferroni correction. Numbers in parentheses in the figure represent the number of samples. **c**, Number of eggs laid per female. Data are shown as mean ± s.e.m. Double asterisk, $P < 0.001$, Student's *t*-test. **d**, Re-mating frequency. Asterisk, $P < 0.0.$, and double asterisk, $P < 0.001$, for comparisons against +/+ (genotype 1); χ^2-test with Bonferroni correction. **e**, Ovipositor extrusions per minute during a ten-min courtship assay with a naive wild-type male. Females used in these assays formed a separate cohort to those in **b**–**d**. Data are mean ± s.e.m. Double asterisk, $P < 0.001$, Student's *t*-test. **f**, Organization of the *SPR* genomic region. *Df(1)Exel6234* is a precise deletion of the interval between P-element insertions *P{XP}d09225* and *P{XP}d00314*. *UAS-SPR-IR1* targets nucleotides 552–852 of the *CG16752-RA* transcript, and *UAS-SPR-IR2* targets nucleotides 869–1,220 (spanning four exons). **g**, Receptivity of virgin females assayed 5 h after injection with either 12 pmol SP (+) or Ringer's solution alone (−). Genotypes were as in **a**, except that all females were virgins. Double asterisk, $P < 0.001$ for comparison to +/+; χ^2-test with Bonferroni correction.

SP is thought to pass from the reproductive tract into the haemolymph, and ultimately to act directly on targets in the central nervous system (CNS)[26,27]. Indeed, staining the adult female CNS with anti-SPR revealed a broad expression on the surface regions of both the brain (Fig. 3e–g) and the ventral nerve cord (VNC, Fig. 3h). This staining was absent or greatly reduced in *SPR* deficiency or RNAi females (Supplementary Fig. 1). Expression was most prominent in ventral regions of the suboesophageal ganglion (SOG), the cervical connective and many nerve roots in the brain and VNC. The restricted staining on the surface of the CNS is consistent with SPR detecting a ligand that circulates in the haemolymph and crosses the blood–brain barrier. It is unlikely to be an artefact caused by poor antibody penetration, because we could reliably detect SPR in central brain regions on ectopic expression of a *UAS-SPR* transgene. Overall, the distribution of SPR concords remarkably well with the reported binding sites of radiolabelled SP applied to whole-female-tissue sections *in vitro*[28]. Intriguingly, a very similar distribution was also observed in the male CNS (Supplementary Fig. 1), although at this point we cannot ascribe any function to SPR in males.

SPR acts in *fru* neurons

Post-mating responses can be induced in virgin females not only by injection of SP (ref. 14) but also by blocking synaptic transmission of neurons that express the sex-specific transcripts of the *fru* gene[10]. We also found that some of the central neurons that express SPR are also positive for *fru*, as revealed by the fru^{GAL4} driver[29] (Fig. 3e–l). In particular, SPR seemed to be expressed in many fru^{GAL4}-positive neurons in the SOG and throughout the VNC. To test whether SPR function is specifically required in *fru* neurons, we used the fru^{GAL4} driver and *UAS-SPR-IR1* to knockdown SPR only in these cells. Just like the *SPR*-deficiency mutants, these females showed normal receptivity as virgins (Fig. 4a), but then laid very few eggs (Fig. 4b) and re-mated at high frequency (Fig. 4c).

To test whether expression in *fru* neurons is also sufficient for the post-mating switch, we introduced fru^{GAL4} and *UAS-SPR* into *SPR*-deficient females. In these females, *SPR* is only expressed in the *fru* neurons, yet we observed complete rescue of the re-mating phenotype (Fig. 4c) and partial but significant rescue of the egg-laying phenotype (Fig. 4b). Together, these RNAi and rescue experiments strongly support the notion[10] that SP triggers the post-mating behavioural switch primarily by modulating the activity of a subset of the *fru* neurons.

Structural and functional conservation of insect SPRs

The post-mating switch in female behaviour is not unique to *D. melanogaster*, but is common to most insect species[6]. Although *SP* genes are difficult to identify outside the Drosophilidae, perhaps because of their small size, we could readily identify putative *SPR* orthologues in most sequenced insect genomes, including *Drosophila pseudoobscura*, the mosquitos *Aedes aegypti* and *Anopheles gambiae*, the moth *Bombyx mori* and the beetle *Tribolium castaneum* (Fig. 5 and Supplementary Fig. 2). More distant relatives can also be detected in *Caenorhabditis elegans*, but potential vertebrate orthologues are less apparent (Fig. 5a).

To test for functional conservation of the insect SPR family, we isolated *SPR* cDNAs from each of these five other insect species and tested them for responses to *D. melanogaster* SP in the CHO cell assay. SP was shown to be a potent activator of the *D. pseudoobscura*, *A. aegypti* and *B. mori* receptors, with EC_{50} values of 4.3 nM, 167 nM and 63 nM, respectively (Fig. 5b–d). These receptors also responded to DUP99B (Fig. 5b–d), but not to any of the eight control peptides, including FMRFamide and hugin-γ. The *A. gambiae* and *T. castaneum* receptors were not activated by either SP or DUP99B

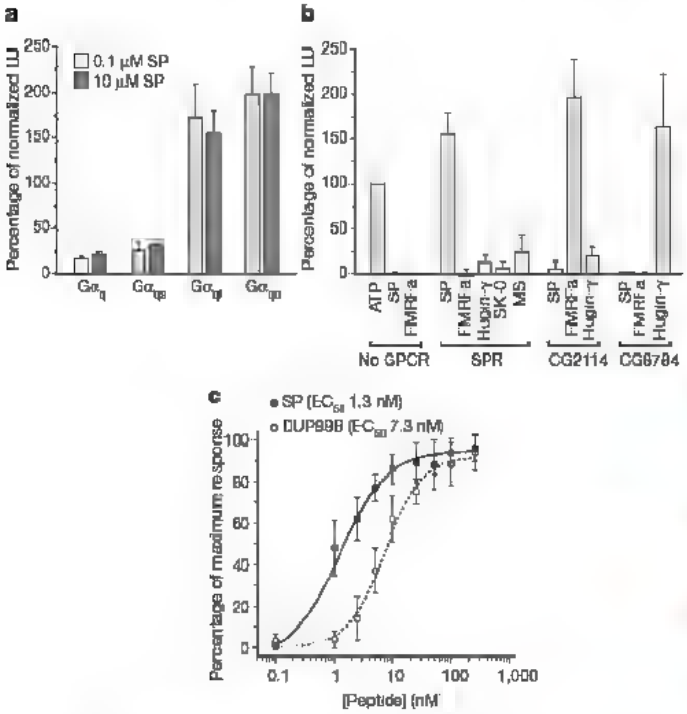


Figure 2 | SPR is a specific SP receptor. **a**, Luminescence (LU) responses of CHO cells expressing SPR, aequorin and one of the three chimaeric G-proteins ($G\alpha_{qs}$, $G\alpha_{qi}$ or $G\alpha_{qo}$) or no additional G protein (endogenous $G\alpha_q$). Cells were treated with either 0.1 μM or 10 μM SP, and responses were normalized against the response to 25 μM ATP. Data are shown as mean ± s.d. ($n = 4–8$). **b**, Luminescence responses of CHO cells expressing the indicated GPCR and aequorin on exposure to various peptide ligands (10 μM), normalized against responses to 25 μM ATP. Cells expressing SPR or no additional GPCR were co-transfected with $G\alpha_{qi}$. Data are shown as mean ± s.d. ($n = 5–8$). FMRFa, FMRFamide; MS, myosuppressin; SK-0, sulfakinin-0. **c**, Dose–response curves of CHO cells expressing SPR, aequorin and $G\alpha_{qi}$, and treated with SP or DUP99B. Each data point is mean ± s.d. ($n = 8$).

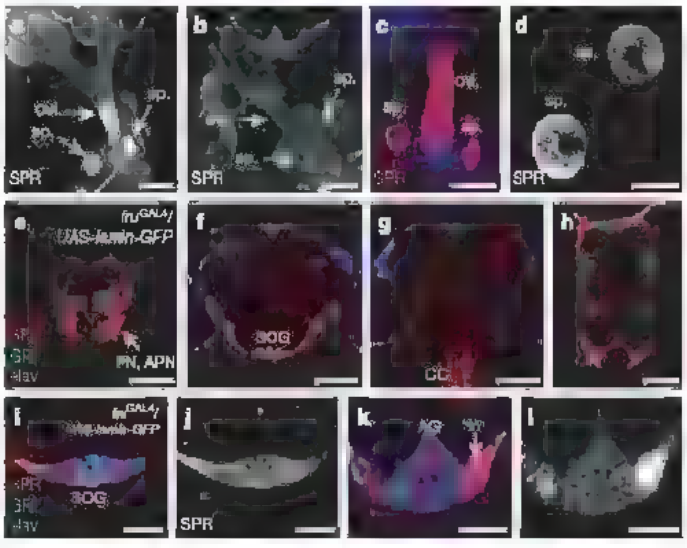


Figure 3 | SPR is expressed in the female reproductive organs and nervous system. **a**, **b**, Reproductive organs of wild-type +/+ (**a**) and *Df(1)Exel6234* homozygous (**b**) females stained with anti-SPR. ovi., oviduct; sp., spermathecae. Scale bar, 200 μm. **c**, **d**, Higher magnification views of wild-type oviduct and spermathecae stained with anti-SPR (red in **c**). The sample in **c** is counterstained with 4,6-diamidino-2-phenylindole (DAPI, blue). Scale bars, 200 μm in **c** and 100 μm in **d**. **e**–**h**, Confocal sections of the brain (**e**–**g**) and ventral nerve cord (**h**) of fru^{GAL4}/*UAS-lamin-GFP* female stained with anti-SPR (red), anti-GFP (green) and anti-ELAV (blue). **e**–**g**, Sections from the anterior, middle and posterior of the brain. APN, accessory pharyngeal nerve; CC, cervical connective; PN, pharyngeal nerve; SOG, suboesophageal ganglion. **e**–**g** are orientated with dorsal up; **h** is orientated with anterior up. Scale bars, 100 μm. **i**–**l**, Higher magnification views of the suboesophageal ganglion (SOG, **i**, **j**) and abdominal ganglion (AG, **k**, **l**), orientated as in **e**–**g**. LN, leg nerve. Scale bars, 25 μm in **i** and **j**, and 50 μm in **k** and **l**.

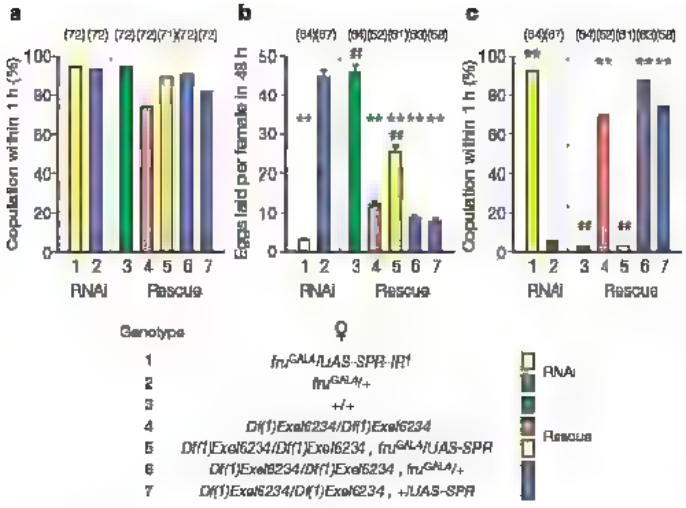


Figure 4 | SPR acts in *fru* neurons. a, b, c, Receptivity (**a**), egg-laying (**b**) and re-mating (**c**) assays for females of the indicated genotype, mated with wild-type males and assayed according to the protocol of Fig. 1a. For the RNAi experiments, the fru^{GAL4} line additionally carried *UAS-Dcr-2* (genotypes 1 and 2). The RNAi (genotypes 1 and 2) and rescue (genotypes 3–7) data are from distinct experimental cohorts. Data in **b** are shown as mean ± s.e.m. Double asterisk, $P < 0.001$ compared to wild-type females (genotypes 2 or 3); ##, $P < 0.001$ compared to deficiency females (genotype 4); Student's *t*-test (**b**) and χ^2 test (**c**)

(Supplementary Fig. 3), possibly because they do not bind the *Drosophila* ligands or were not functionally expressed in CHO cells. Nonetheless, the structural and functional conservation of *SPR* genes from *Drosophila*, *Aedes* and *Bombyx* (Fig. 5), together with the observation that *D. melanogaster* SP can induce post-mating responses in the moth *Helicoverpa armigera*[30,31], indicates that the family of receptors we have identified are likely to mediate post-mating changes in female reproductive behaviour in many different insect orders.

Conclusions

The data presented here provide strong evidence that SPR is a receptor for SP, and that activation of SPR in *fru* neurons induces the switch to post-mating reproductive behaviour. Our identification of SPR is the critical first step in explaining this behavioural switch at the molecular, cellular and circuit levels. Furthermore, because SPR is highly conserved across insect species, it provides the basis for cellular assays to identify SP-like activities in other species, and to develop new approaches for controlling the reproductive or host-seeking behaviours of various agricultural pests and human disease vectors.

METHODS SUMMARY

UAS-SPR-IR1 was obtained from the Vienna *Drosophila* RNAi Center[11]. *UAS-SPR-IR2* was prepared by cloning a 352 base-pair (bp) fragment from the RE15519 cDNA as an inverted repeat into a *UAS* vector[32] modified for integration using the φC31 system[35]. *UAS-SPR* was obtained by cloning the full open reading frame of RE15519 into a similar vector. *Df(1)Exel6234* (ref. 12) was obtained from the Bloomington *Drosophila* Stock Center and was backcrossed into a wild-type Canton S background. *SP* null mutants were SP^0/Δ^{130} mutants[9]. The *elav-GAL4* (ref. 34) and fru^{GAL4} (ref. 29) stocks used for the RNAi experiments additionally carried *UAS-Dcr-2* (ref. 11). Virgin males and females were collected at eclosion and were aged individually for five days, or in groups of 10–15 for four days, respectively. Canton S was used as wild type. SP injections into the abdomens of female virgins were performed as described[34].

CHO-K1 cells were transiently transfected, and luminescent signals were measured as described previously[35]. All peptides were synthesized in-house using the 9-fluorenyl-methoxycarbonyl (FMOC) strategy on an ABI 433A Peptide Synthesizer and were purified by high-performance liquid chromatography (HPLC). Anti-SPR antisera were raised in rabbits against the predicted N-terminal 21 amino acids of the mature SPR, and were cleared by incubating with *Df(1)Exel6234* embryos. Antibody stainings and confocal microscopy were performed essentially as described previously[29].

SPR orthologues were predicted by TBLASTN and Genscan analyses, and the complete open reading frame was amplified by RT–PCR on RNA extracted from the relevant species. *D. pseudoobscura* was obtained from the Tucson *Drosophila* Stock Center, *T. castaneum* from G. Bucher, and *B. mori* from D. Zitnan. Frozen

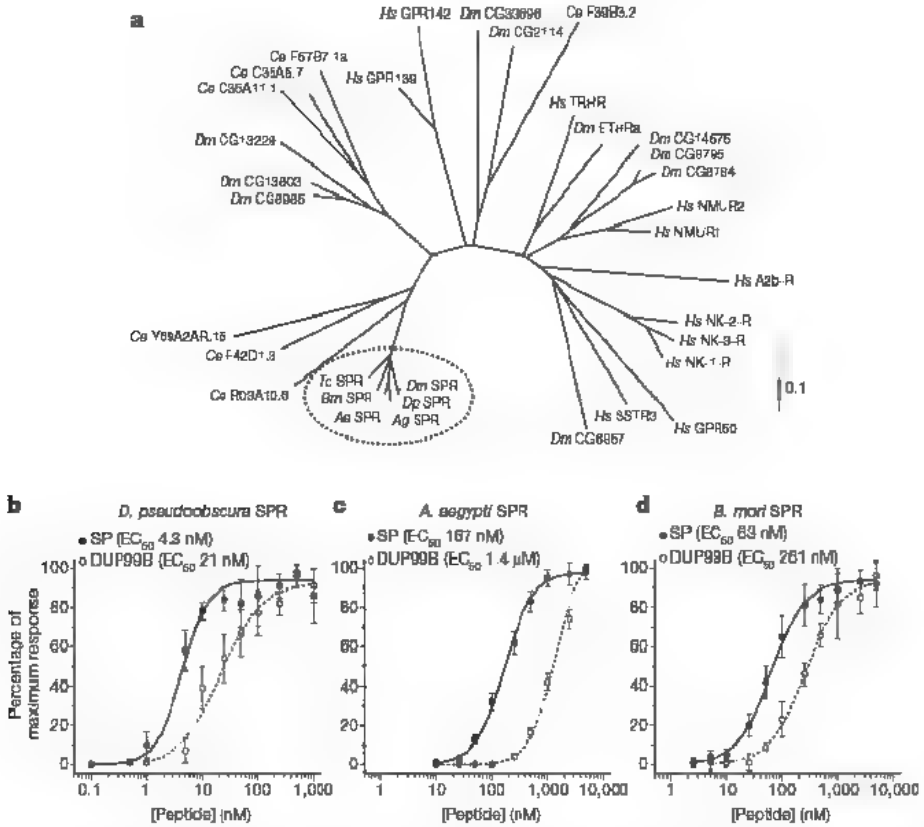


Figure 5 | Structural and functional conservation of insect SPRs. **a**, Phylogenetic tree of predicted insect SPRs and related *Drosophila* (*Dm*), *C. elegans* (*Ce*) and human (*Hs*) GPCRs. Scale bar, 0.1 amino acid replacements per site. **b–d**, Dose–response curves of CHO cells expressing insect SPRs, aequorin and $G\alpha_{qi}$ treated with *D. melanogaster* SP or DUP99B. Each data point is mean ± s.d. ($n = 6$).

stocks of *A. aegypti* and *A. gambiae* were obtained from the MR4 Resource Center

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 21 August; accepted 19 November 2007.
Published online 9 December 2007.

1. Arthur, B. I. Jr, Jallon, J. M., Caflisch, B., Choffat, Y. & Nothiger, R. Sexual behaviour in *Drosophila* is irreversibly programmed during a critical period. *Curr. Biol.* 8, 1187–1190 (1998)
2. Morris, J. A., Jordan, C. L. & Breedlove, S. M. Sexual differentiation of the vertebrate nervous system. *Nature Neurosci.* 7, 1034–1039 (2004)
3. Munday, P. L., Buston, P. M. & Warner, R. R. Diversity and flexibility of sex-change strategies in animals. *Trends Ecol. Evol.* 21, 89–95 (2006).
4. Demir, E. & Dickson, B. J. *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell* 121, 785–794 (2005)
5. Manoli, D. S. *et al.* Male-specific *fruitless* specifies the neural substrates of *Drosophila* courtship behaviour. *Nature* 436, 395–400 (2005).
6. Gillott, C. Male accessory gland secretions: modulators of female reproductive physiology and behavior. *Annu. Rev. Entomol.* 48, 163–184 (2003)
7. Chen, P. S. *et al.* A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell* 54, 291–298 (1988)
8. Chapman, T. *et al.* The sex peptide of *Drosophila melanogaster* female post-mating responses analyzed by using RNA interference. *Proc. Natl Acad. Sci. USA* 100, 9923–9928 (2003)
9. Liu, H. & Kubli, E. Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* 100, 9929–9933 (2003).
10. Kvitsiani, D. & Dickson, B. J. Shared neural circuitry for female and male sexual behaviours in *Drosophila*. *Curr. Biol.* 16, R355–R356 (2006).
11. Dietzl, G. *et al.* A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 448, 151–156 (2007)
12. Parks, A. L. *et al.* Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. *Nature Genet.* 36, 288–292 (2004).
13. Santel, A., Winhauer, T., Blumer, N. & Renkawitz-Pohl, R. The *Drosophila don juan* (*dj*) gene encodes a novel sperm specific protein component characterized by an unusual domain of a repetitive amino acid motif. *Mech. Dev.* 64, 19–30 (1997)
14. Schmidt, T., Choffat, Y., Klauser, S. & Kubli, E. The *Drosophila melanogaster* Sex-peptide: a molecular analysis of structure–function relationships. *J. Insect Physiol.* 39, 361–368 (1993).
15. Le Poul, E., Hisada, S., Mizuguchi, Y., Dupriez, V. J., Burgeon, E. & Detheux, M. Adaptation of aequorin functional assay to high throughput screening. *J. Biomol. Screen.* 7, 57–65 (2002)
16. Harshman, L. G., Loeb, A. M. & Johnson & B. A., Ecdysteroid titers in mated and unmated *Drosophila melanogaster* females. *J. Insect Physiol.* 45, 571–577 (1999).
17. Chapman, T., Choffat, Y., Lucas, W. E., Kubli, E. & Partridge, L. Lack of response to sex-peptide results in increased cost of mating in *dunce Drosophila melanogaster* females. *J. Insect Physiol.* 42, 1007–1015 (1996)
18. Conklin, B. R., Farfel, Z., Lustig, K. D., Julius, D. & Bourne, H. R. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. *Nature* 363, 274–276 (1993)
19. Conklin, B. R. *et al.* Carboxyl-terminal mutations of $G_q\alpha$ and $G_s\alpha$ that alter the fidelity of receptor activation. *Mol. Pharmacol.* 50, 885–890 (1996).
20. Meeusen, T. *et al.* Identification in *Drosophila melanogaster* of the invertebrate G protein-coupled FMRFamide receptor. *Proc. Natl Acad. Sci. USA* 99, 15363–15368 (2002)
21. Cazzamali, G. & Grimmelikhuijzen, C. J. Molecular cloning and functional expression of the first insect FMRFamide receptor. *Proc. Natl Acad. Sci. USA* 99, 12073–12078 (2002)
22. Park, Y., Kim, Y.-J. & Adams, M. E. Identification of G protein-coupled receptors for *Drosophila* PRXamide peptides, CCAP, corazonin, and AKH supports a theory of ligand–receptor coevolution. *Proc. Natl Acad. Sci. USA* 99, 11423–11428 (2002)
23. Rosenkilde, C. *et al.* Molecular cloning, functional expression, and gene silencing of two *Drosophila* receptors for the *Drosophila* neuropeptide pyrokinin-2. *Biochem. Biophys. Res. Commun.* 309, 485–494 (2003)
24. Saudan, P. *et al.* Ductus ejaculatorius peptide 99B (DUP99B), a novel *Drosophila melanogaster* sex-peptide pheromone. *Eur. J. Biochem.* 269, 989–997 (2002).
25. Bloch Qazi, M. C., Heifetz, Y. & Wolfner, M. F. The developments between gametogenesis and fertilization: ovulation and female sperm storage in *Drosophila melanogaster*. *Dev. Biol.* 256, 195–211 (2003)
26. Kubli, E. Sex-peptides: seminal peptides of the *Drosophila* male. *Cell. Mol. Life Sci.* 60, 1689–1704 (2003)
27. Peng, J. *et al.* Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*. *Curr. Biol.* 15, 207–213 (2005)
28. Ottiger, M., Soller, M., Stocker, R. F. & Kubli, E. Binding sites of *Drosophila melanogaster* sex peptide pheromones. *J. Neurobiol.* 44, 57–71 (2000).
29. Stockinger, P., Kvitsiani, D., Rotkopf, S., Tirian, L. & Dickson, B. J. Neural circuitry that governs *Drosophila* male courtship behavior. *Cell* 121, 795–807 (2005)
30. Fan, Y., Rafaeli, A., Gileadi, C., Kubli, E. & Applebaum, S. W. *Drosophila melanogaster* sex peptide stimulates juvenile hormone synthesis and depresses sex pheromone production in *Helicoverpa armigera*. *J. Insect Physiol.* 45, 127–133 (1999)
31. Fan, Y. *et al.* Common functional elements of *Drosophila melanogaster* seminal peptides involved in reproduction of *Drosophila melanogaster* and *Helicoverpa armigera* females. *Insect Biochem. Mol. Biol.* 30, 805–812 (2000)
32. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415 (1993)
33. Groth, A. C., Fish, M., Nusse, R. & Calos, M. P. Construction of transgenic *Drosophila* by using the site-specific integrase from phage phiC31. *Genetics* 166, 1775–1782 (2004).
34. Luo, L., Liao, Y. J., Jan, L. Y. & Jan, Y. N. Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.* 8, 1787–1802 (1994)
35. Kim, Y.-J. *et al.* Corazonin receptor signaling in ecdysis initiation. *Proc. Natl Acad. Sci. USA* 101, 6704–6709 (2004)

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank A. Scheiffer for preparation of the phylogenetic tree, R. Fuchs and G. Krssakova for technical assistance, M. Calos, K-C. Su and S. Oppel for φC31 reagents, and G. Bucher and D. Zitnan for insect stocks. We particularly thank E. Kubli for many reagents and discussions. Y-J.K. was supported by a Lise Meitner postdoctoral fellowship from the Austrian Science Fund, and C.R. was supported by an EMBO postdoctoral fellowship and an Advanced Researcher fellowship from the Swiss National Science Foundation. Basic research at the IMP is funded by Boehringer Ingelheim GmbH.

Author Contributions N.Y. and C.R. identified *D. melanogaster SPR* in the RNAi screen, N.Y. performed the initial molecular analysis and all behavioural assays, and Y.-J.K. performed the cellular assays and immunohistochemistry and cloned *SPR* orthologues from other insects. B.J.D. supervised the project and wrote the manuscript together with N.Y. and Y.-J.K.

Author Information Nucleotide sequences and translations of the reported SPRs have been deposited in the GenBank database under the following accession numbers: *D. pseudoobscura*, EU106873; *A. aegypti*, EU106874; *A. gambiae*, EU106875; *B. mori*, EU106876; and *T. castaneum*, EU106877. Reprints and permissions information is available at www.nature.com/reprints
Correspondence and requests for materials should be addressed to B.J.D. (dickson@imp.ac.at)

LETTERS

A young massive planet in a star–disk system

J. Setiawan[1], Th. Henning[1], R. Launhardt[1], A. Müller[1], P. Weise[1] & M. Kürster[1]

There is a general consensus that planets form within disks of dust and gas around newly born stars[1,2]. Details of their formation process, however, are still a matter of ongoing debate. The timescale of planet formation remains unclear, so the detection of planets around young stars with protoplanetary disks is potentially of great interest. Hitherto, no such planet has been found. Here we report the detection of a planet of mass $(9.8\pm3.3)M_{\rm Jupiter}$ around TW Hydrae (TW Hya), a nearby young star with an age of only 8–10 Myr that is surrounded by a well-studied circumstellar disk. It orbits the star with a period of 3.56 days at 0.04 AU, inside the inner rim of the disk. This demonstrates that planets can form within 10 Myr, before the disk has been dissipated by stellar winds and radiation.

With the discovery of the first planet orbiting another Sun-like star[3], our understanding of planet formation has experienced a renaissance. The vast majority of exoplanets have been discovered with the radial velocity (RV) technique[4]. This method is most sensitive to giant planets on short-period orbits and it works best with non-active solar-like stars. Other techniques with different detection biases are currently emerging (for example, transit photometry, astrometry, direct imaging), but the RV method still remains the most successful technique for detecting exoplanets. Correspondingly, our picture of extrasolar planetary systems is still dominated by the detection biases of this method.

Planets form from dust and gas in circumstellar disks around young stars[1,2]. The growth from micrometre-sized dust grains to planetary embryos through collisions is believed to be the key mechanism leading to the formation of planetary cores. As these cores grow, they eventually become massive enough to accrete gas from the disk. Alternatively, giant planets are also proposed to form directly via gravitational instabilities in the disk[5].

One of the most important timescales for planet formation is the disk dispersal time. From observations of near-infrared excess[6,7] and millimetre-wavelength emission[8] of young stars it has been concluded that circumstellar disks dissipate within about 10 Myr after the star formation. The formation of planets from disk material must occur in this time window. Until now, no planet has been detected by RV surveys around a star younger than 100 Myr old. The only known young planet was detected by direct imaging at 55 AU from the brown dwarf 2MASS1207 (ref. 9). The main reason for this lack of detections is that young stars were systematically excluded from large RV surveys because they usually exhibit high levels of stellar activity. However, when carefully analysing and characterizing all activity effects, it is also possible to detect planets around young stars with the RV technique[10]. The young star TW Hya (see Supplementary Table 1) is surrounded by a circumstellar disk that has been studied by using a wide variety of different observing techniques and analysis methods[11–19] (Fig. 1). From Hubble Space Telescope observations the disk was found to be oriented almost face-on[11].

We acquired high-resolution spectroscopic measurements of TW Hya with the Fibre-fed Extended Range Optical Spectrograph (FEROS)[20] at the 2.2 m Max-Planck-Gesellschaft/European Southern Observatory (MPG/ESO) telescope (La Silla Observatory). Data reduction and RV determination procedures for FEROS data are described in ref. 21. For the RV computations we excluded spectral regions that contain strong emission lines (such as Ca II H&K, Hβ, He I, Na I and Hα). The RV measurements are shown in Fig. 2 and listed in Supplementary Table 2. The sine-fitting periodogram of the entire RV data set shows three distinct significant periods (Fig. 3). The first and most pronounced signal at $P = 3.56$ days has a false alarm probability (FAP) of 10^{-14}. This period corresponds to the regular sinusoidal variation shown in Fig. 2. Further signals are found at 0.78 and 1.39 days. However, after subtracting the 3.56-day period from the data and recomputing the periodogram for the residual RVs, these two peaks disappear. Hence, they are aliases. Other peaks that appear in the periodogram of the residual RVs have high FAPs and are not significant.

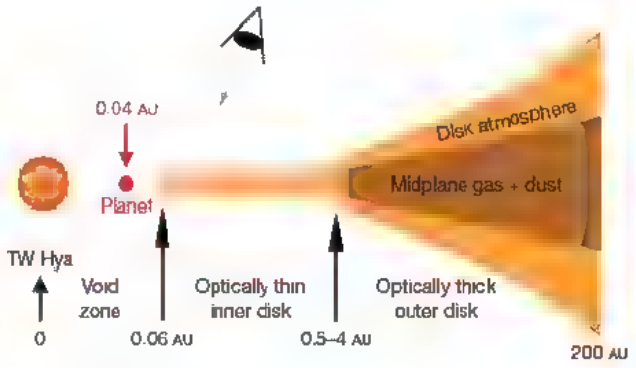


Figure 1 | A pictographic sketch of the TW Hya system. The star, the disk with a central hole, and the newly discovered planet are shown. The circumstellar disk around TW Hya is almost face-on[11]. Recent measurements yielded a disk inclination of $7^\circ \pm 1^\circ$ (ref. 12). Ref. 13 concluded that an optically thin inner-disk zone void of large dust particles would model the complete spectral energy distribution well. The relatively sharp transition to the outer optically thick and geometrically flared disk was predicted to be located at ~4 AU. They[13] speculated about the existence of a giant planet in this region that could have caused the clearing. The existence of such a transition at ~4 AU was confirmed by millimetre interferometric observations from 7 mm Very Large Array observations[14]. Mid-infrared interferometric observations[15] showed that the transition between cleared inner and optically thick outer disk must occur at radii between 0.5 to 0.8 AU. Mid-infrared observations are more sensitive to smaller dust particles compared to the millimetre observations, which may explain the difference. On the other hand, CO gas emission from a region inside 1.0 AU has been detected, showing that this region is not completely void of material[16,17]. From Keck near-infrared interferometric observations it was concluded that the inner edge of the disk is located at about 0.06 ± 0.01 AU from the star[18]. This is somewhat larger than the dust sublimation radius, but the precise value depends critically on the assumed dust properties. The authors[18] speculated that magnetospheric accretion may be responsible for this truncation. From the Hα line and short-wavelength continuum excess, ref. 19 derived a mass accretion rate from the disk onto the star of $(\sim 5 \times 10^{-10}) M_{\rm Sun}$ per year and concluded that TW Hya is at the end of its accretion stage. The planet at 0.04 AU marked here is the result of our RV monitoring survey.

[1]Max-Planck-Institut für Astronomie, Heidelberg, D-69117 Germany

RV variations can be induced either by an orbiting companion, rotational modulation due to starspots, or nonradial pulsations. In the case of a companion, all spectral lines move simultaneously without affecting the line profile. In the case of rotational modulation and nonradial pulsations, the integral line shapes will vary and cause changes in the measured effective RV. To verify the nature of the observed RV variations, it is therefore mandatory to analyse the stellar activity indicators.

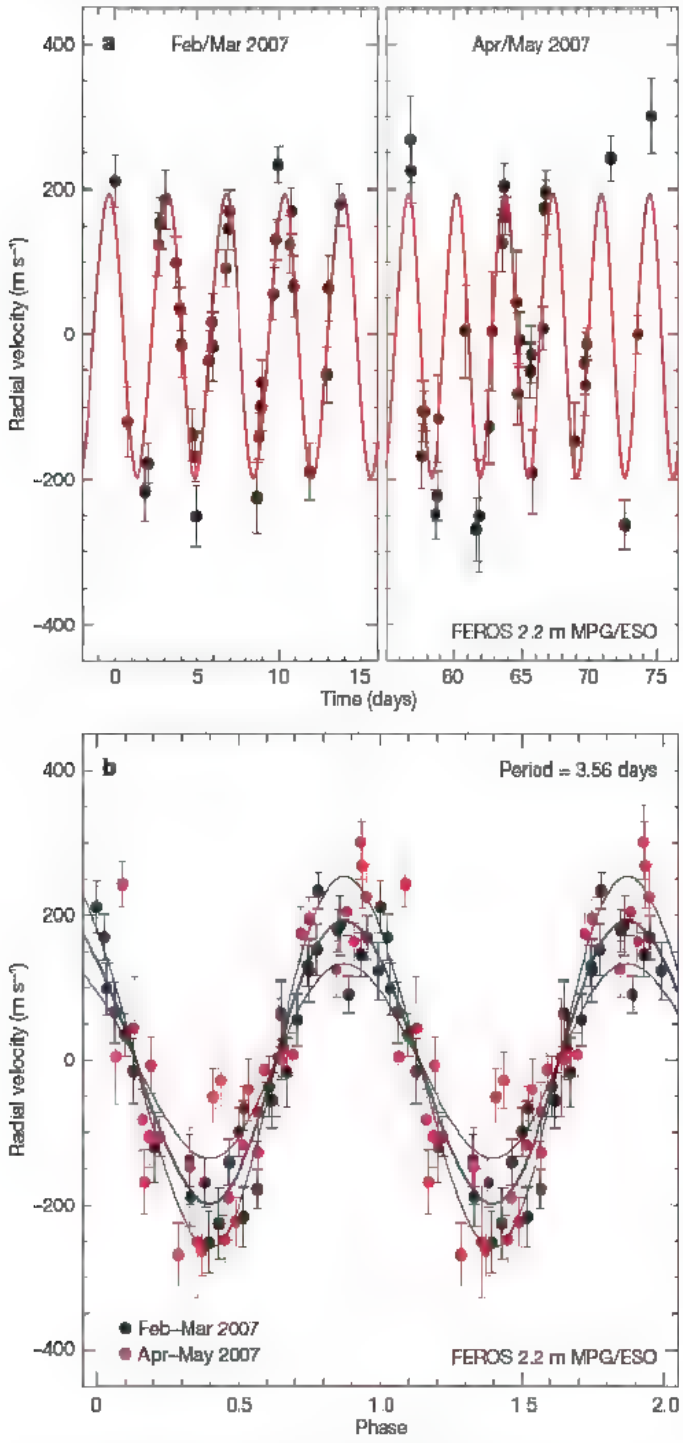


Figure 2 | Radial velocity variation of TW Hya. a, The RVs were obtained during two observing runs with 12 consecutive nights (between 28 February and 12 March 2007) and 20 consecutive nights (24 April to 13 May 2007). With typically three spectra per night, we sampled possible variability periods from about 1 to 12 days. For the RV calculations we used a cross-correlation technique, in which about 1,300 spectral lines were cross-correlated with a numerical template. The error bars are standard errors of the mean RV value. The typical accuracy of the individual RV is about 40 m s^{-1}, which is mostly due to the rapid rotation and activity of TW Hya. For comparison, the typical accuracy achieved with FEROS for quiet and slow rotating solar-type stars is about 5 m s^{-1}. The solid line shows a keplerian fit with a period of 3.56 days. The scatter of the data points around this curve (residuals) is probably due to stellar activity. **b**, The phase-folded (with $P = 3.56$ days) RV curve (blue line) of the planet around TW Hya. This periodic variation is stable within the observation time window. The amplitude of RV variation is 196 ± 61 m s^{-1}. The black lines represent the uncertainty of ± 61 m s^{-1} below and above the blue curve.

Table 1 | Orbital parameters for TW Hya b

Primary mass	$(0.7 \pm 0.1) M_{Sun}$
Orbital period	(3.56 ± 0.02) days
Offset RV	$(12{,}420.7 \pm 4.1)$ m s^{-1}
RV semi-amplitude	(196 ± 61) m s^{-1}
Inclination angle	$(7 \pm 1)°$
Eccentricity	0.04 ± 0.03
Periastron longitude	$(105 \pm 27)°$
Reduced χ^2	3.32
Minimum companion mass	$(1.2 \pm 0.4) M_{Jupiter}$
True companion mass	$(9.8 \pm 3.3) M_{Jupiter}$
Orbital semi-major axis	(0.041 ± 0.002) AU

The 3.56-day RV variation appears to be regular during both observing periods. The phase-folded RV curve reveals very clearly the nearly sinusoidal variation. We find that this period is neither correlated with photometric variations nor with any stellar activity indicators. The bisector analysis of the line profile asymmetries confirms that there is no significant correlation between the 3.56-day period and the stellar activity (Fig. 4). The most probable explanation of the 3.56-day RV variation is therefore the presence of a companion orbiting TW Hya. We calculated an orbital solution using a keplerian fit to the RV variation (Table 1) and derived a minimum companion mass of $(1.2 \pm 0.4) M_{Jupiter}$. Assuming that the companion orbits the star in the plane of the disk ($i = 7° \pm 1°$) and also taking into account all other uncertainties, we computed a true companion mass of

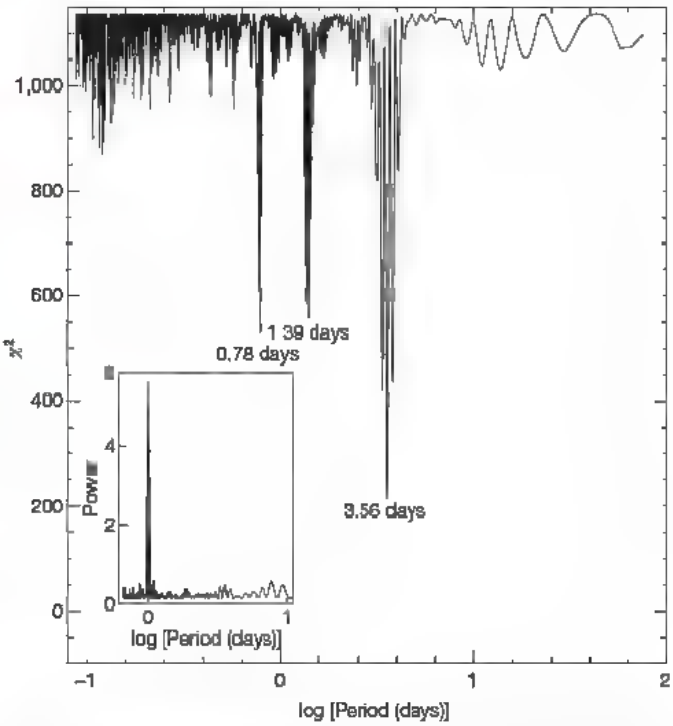


Figure 3 | Sine-fitting periodogram of RV variation. A period analysis was performed using both a sine-fitting routine minimizing χ^2 (ref. 29), and the Lomb–Scargle periodogram (Supplementary Fig. 1). The window function is displayed in the inset and has only a single peak at $P = 1$ day. For the sine-fitting we used the bootstrap randomization method[30] to determine FAPs—that is, the probability that a value of χ^2 as small as (or smaller than) the optimum value found for the data was obtained purely by chance. We found three significant χ^2 minima at distinct periods. The first and most pronounced χ^2 minimum at $P = 3.56$ days has a FAP = 10^{-14}. Further χ^2 minima are seen at 0.78 and 1.39 days, both with FAP = 10^{-5}. We calculated the residual RVs by subtracting the 3.56-day periodicity. In the sine-fitting periodogram of the residual RVs, the 0.78 and 1.39-day periods are no longer seen in the periodogram. Thus, they are aliases.

$(9.8 \pm 3.3)M_{Jupiter}$. This mass range clearly qualifies the companion as a giant planet. The orbit of the planet is almost circular and has a semimajor axis of (0.041 ± 0.002) AU, placing it inside the inner hole of the disk.

Several authors have tried to derive the rotation period of TW Hya from photometric observations[22,23] (Supplementary Table 3). However, classical T Tauri stars do not easily reveal their rotation periods, because random variations due to accretion and related short-lived hot spots tend to mask their periodic behaviour. Phase-coherent periodic modulations may only persist for a few rotation cycles.

We investigated all available activity indicators in the spectra, including the Hα emission line (Supplementary Fig. 2). We selected several short time intervals during which obvious periodic variations are persistent (Supplementary Fig. 3). We then computed the periodicities independently for each subset. For the residual RVs and the Ca II activity index we found periods of 1.4, 1.7 and 2.3 days with typical uncertainties of ±0.3 day. Other activity indicators (for example, Hβ, He I and effective temperature (T_{eff}) variation) yielded similar results. Assuming that all individual values represent uncertain measurements of the same underlying period, we attribute the weighted mean to the stellar rotation period, that is $P_{rot} = 1.6 \pm 0.3$ days. This period is in agreement with the photometric variability reported by other authors. Combining P_{rot} with the stellar radius and rotation velocity of TW Hya, we derived an inclination angle of the stellar rotation axis of 14° ± 4°, comparable with that of ref. 24. When we analysed the bisector velocity spans and bisector curvatures we found no significant correlation with the RVs (Fig. 4), confirming that the 3.56-day RV period is not caused by activity-related lineshape variations.

There is one activity indicator that varies with another significant period, at 9.05 days: the equivalent width of the Hα. The

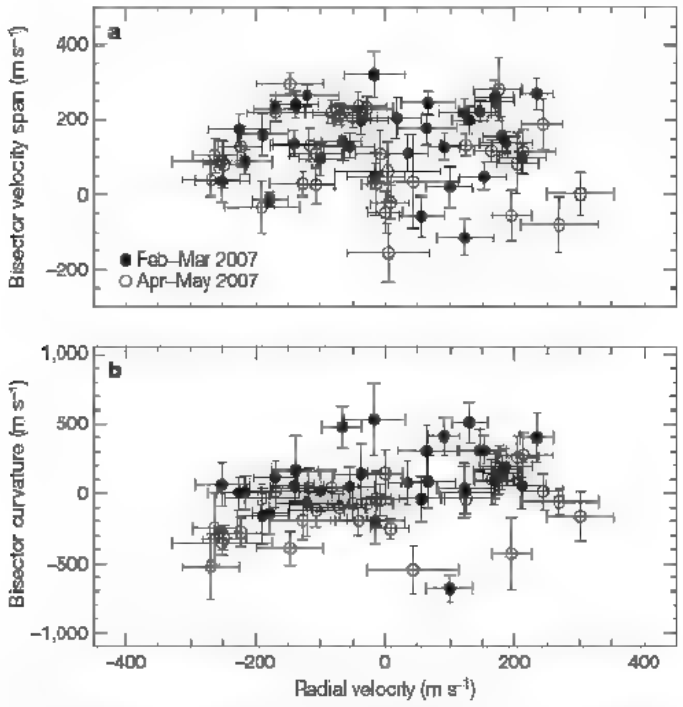


Figure 4 | Bisector analysis of line profile asymmetry. We used a cross-correlation technique, using several hundred spectral lines of TW Hya. We measured the bisector velocity spans (**a**) and bisector curvatures (**b**), which are well known as excellent stellar activity indicators. **a**, Bisector velocity span versus RV for the entire data set. There is no significant correlation (correlation coefficient ~0.2), indicating that the 3.56-day RV variation is not caused by the line profile changes. **b**, The bisector curvature does not show a significant correlation with the RV (correlation coefficient ~0.3), confirming that stellar activity is not responsible for the observed 3.56-day RV variation. The error bars are the standard mean errors of the mean bisector velocity span/curvature, computed from the bisectors of each echelle order

interpretation of this variability is still inconclusive, but it is probably related to a phenomenon in the disk. The corresponding keplerian radius would be ~0.07 AU, which is very close to the inner edge of the disk[18].

The detection of a young (8–10 Myr) and massive planet with $9.8M_{Jupiter}$ on a 0.04 AU orbit around TW Hya provides important constraints on theories of planet formation and migration. It gives a real upper limit to the timescales of planet formation and migration that is not based on purely statistical arguments. Furthermore, this is the first direct observational link (to our knowledge) between a circumstellar disk and a newly formed planet, thus justifying calling such disks 'protoplanetary'.

In the period–mass distribution of known exoplanets[4], massive planets with masses $>2M_{Jupiter}$ seem to be rare at semi-major axes of less than 0.6 AU. However, such statistics are based mostly on RV data, which provide only lower limits to the planet masses. When we use $msini$ ($1.2M_{Jupiter}$) instead of the true mass (where m is the mass and i is the the inclination angle), TW Hya b falls in the middle of the period–mass distribution for RV planets. These facts show that our current understanding of the population of exoplanets is still biased by the detection method.

Models that assume the formation of planets beyond the snowline and include migration and accretion allow the formation of massive planets. However, it is still not clear whether a planet as massive as $9.8M_{Jupiter}$ could have formed through core accretion[25,26], or whether gravitational instabilities in the disk must have been involved[27].

A massive planet like TW Hya b would open a gap in the disk and undergo type II migration on a typical timescale of 10^5 years[28]. TW Hya b may have formed and started its migration path close to the present inner edge of the optically thick disk at 1–4 AU. We can even speculate that the planet is responsible for the clearing of the inner disk through the accretion of the gas. Its inward migration came to a halt when the planet entered the gas-free zone at the inner edge of the optically thin disk at ~0.06 AU. This inner hole can have formed, for example, by disk dispersal processes or by the stellar magnetosphere.

The detection of TW Hya b opens up the possibility of directly connecting the disk evolution and planet formation processes. It is the ideal system to test numerical simulations of planet core formation, migration and accretion.

Received 6 July; accepted 18 October 2007.

1. Safronov, V. S. The protoplanetary cloud and its evolution. *Sov. Astron.* 10, 650–658 (1967)
2. Pollack, J. B. *et al.* Formation of the giant planets by concurrent accretion of solids and gas. *Icarus* 124, 62–85 (1996).
3. Mayor, M. & Queloz, D. A. Jupiter-mass companion to a solar-type star. *Nature* 378, 355–359 (1995).
4. *The Extrasolar Planet Encyclopedia* (www.exoplanet.eu)
5. Boss, A. P. Gas giant protoplanet formation: disk instability models with thermodynamics and radiative transfer. *Astrophys. J.* 563, 367–373 (2001).
6. Hatsch, K. E. Jr, Lada, E. A. & Lada, C. J. Disk frequencies and lifetimes in young clusters. *Astrophys. J.* 553, L153–L156 (2001).
7. Cieza, L. *et al.* The Spitzer c2d Survey of weak-line T-Tauri stars. II. New constraints on the timescale for planet building. *Astrophys. J.* 667, 308–328 (2007)
8. Carpenter, J. M. *et al.* Evolution of cold circumstellar dust around solar-type stars. *Astron. J.* 129, 1049–1062 (2005)
9. Chauvin, G. *et al.* Giant planet companion to 2MASSW J1207334–393254. *Astron. Astrophys.* 438, L25–L28 (2005).
10. Setiawan, J. *et al.* Evidence for a planetary companion around a nearby young star. *Astrophys. J.* 660, L145–L148 (2007)
11. Krist, J. E. *et al.* WFPC2 images of a face-on disk surrounding TW Hydrae. *Astrophys. J.* 538, 793–800 (2000).
12. Qi, C. *et al.* Imaging the disk around TW Hydrae with the submillimeter array. *Astrophys. J.* 616, L11–L14 (2004)
13. Calvet, N. *et al.* Evidence for a developing gap in a 10 Myr old protoplanetary disk. *Astrophys. J.* 568, 1008–1016 (2002)
14. Hughes, A. M. *et al.* An inner hole in the disk around TW Hydrae resolved in 7 mm dust emission. *Astrophys. J.* 664, 536–542 (2007)
15. Ratzka, T. *et al.* High spatial resolution mid-infrared observations of the low-mass young star TW Hya. *Astron. Astrophys.* 471, 173–185 (2007).
16. Rettig, T. W. *et al.* Discovery of CO gas in the inner disk of TW Hydrae. *Astrophys. J.* 616, L163–L166 (2004)

17 Salyk, C., Blake, G. A., Boogert, A. C. A. & Brown, J. M. Molecular gas in the inner 1 AU of the TW Hya and GM Aur transitional disks. *Astrophys. J.* 655, L105–L108 (2007)
18. Eisner, J. A., Chiang, E. I. & Hillenbrand, L. A. Spatially resolving the inner disk of TW Hydrae. *Astrophys. J.* 637, L133–L136 (2006)
19 Muzerolle, J. *et al.* Disk accretion in the 10 Myr old T Tauri stars TW Hydrae and Hen 3–600A. *Astrophys. J.* 535, L47–L50 (2000)
20 Kaufer, A. *et al.* Performance report on FEROS, the new fiber-linked echelle spectrograph at the ESO 1.52-m telescope. In *Proc. SPIE Optical and IR Telescope Instrumentation and Detectors* 4008, 459–466 (2000)
21 Setiawan, J. *et al.* Precise radial velocity measurements of G and K giants. First results. *Astron. Astrophys.* 397, 1151–1159 (2003)
22 Herbst, W. & Koret, D. L. Rotation periods of four T Tauri stars. *Astron. J.* 96, 1949–1955 (1988).
23 Mekkaden, M. V. Photometric and polarimetric variability of the isolated T Tauri star TW Hydrae. *Astron. Astrophys.* 340, 135–142 (1998).
24 Alencar, S. H. P. & Batalha, C. Variability of southern T Tauri stars. II. The spectral variability of the classical T Tauri star TW Hydrae. *Astrophys. J.* 571, 378–393 (2002).
25. Alibert, Y., Mordasini, C., Benz, W. & Winisdoerffer, C. Models of giant planet formation with migration and disc evolution. *Astron. Astrophys.* 434, 343–353 (2005)
26. Matsuo, T., Shibai, H., Ootsubo, T. & Tamura, M. Planet formation scenarios revisited: core accretion versus disk instability. *Astrophys. J.* 662, 1282–1292 (2007).
27 Boss, A. Evolution of the solar nebula. VII. Planet formation and survival of protoplanets formed by disk instability. *Astrophys. J.* 629, 535–548 (2005).
28. Papaloizou, J. C. B. Disk-planet interaction during planet formation. *Protostars Planets* 5, 655–668 (2007).
29. Cumming, A., Marcy, G. W. & Butler, R. P. The Lick planet search: detectability and mass thresholds. *Astrophys. J.* 526, 890–915 (1999).
30. Kürster, M., Schmitt, J. H. M. M., Cutispoto, G. & Dennerl, K. ROSAT and AB Doradus: the first five years. *Astron. Astrophys.* 320, 831–839 (1997)

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the 2.2 m MPG/ESO La Silla team, especially P. Francois, B. Conn, M. Stefanon, O. Schütz, M. Morell and A. Gonzales for their help during the observations. We thank W. Herbst for constructive discussion and providing the supporting data.

Author Contributions The observations were carried out by J.S. and A.M.; T.H. and R.L. were responsible for the project planning. The data analysis was done by J.S., A.M., P.W. and M.K.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.S. (setiawan@mpia.de) or R.L. (rlau@mpia.de)

LETTERS

Magnetic monopoles in spin ice

C. Castelnovo[1], R. Moessner[1,2] & S. L. Sondhi[3]

Electrically charged particles, such as the electron, are ubiquitous. In contrast, no elementary particles with a net magnetic charge have ever been observed, despite intensive and prolonged searches (see ref. 1 for example). We pursue an alternative strategy, namely that of realizing them not as elementary but rather as emergent particles—that is, as manifestations of the correlations present in a strongly interacting many-body system. The most prominent examples of emergent quasiparticles are the ones with fractional electric charge $e/3$ in quantum Hall physics[2]. Here we propose that magnetic monopoles emerge in a class of exotic magnets known collectively as spin ice[3–5]: the dipole moment of the underlying electronic degrees of freedom fractionalises into monopoles. This would account for a mysterious phase transition observed experimentally in spin ice in a magnetic field[6,7], which is a liquid–gas transition of the magnetic monopoles. These monopoles can also be detected by other means, for example, in an experiment modelled after the Stanford magnetic monopole search[8].

Spin-ice materials are characterized by the presence of magnetic moments $\boldsymbol{\mu}_i$ residing on the sites i of a pyrochlore lattice (depicted in Fig. 1). These moments are constrained to point along their respective local Ising axes $\hat{\mathbf{e}}_i$ (the diamond lattice bonds in Fig. 1), and they can be modelled as Ising spins $\boldsymbol{\mu}_i = \mu S_i \hat{\mathbf{e}}_i$, where $S_i = \pm 1$ and $\mu = |\boldsymbol{\mu}_i|$. For the spin-ice compounds discussed here, $Dy_2Ti_2O_7$ and $Ho_2Ti_2O_7$, (where Dy is dysprosium and Ho is holmium) the magnitude μ of the magnetic moments equals approximately ten Bohr magnetons ($\mu \approx 10\mu_B$). The thermodynamic properties of these compounds are known to be described with good accuracy by an energy term that accounts for the nearest-neighbour exchange and the long-range dipolar interactions[9,10] (for a review of spin ice, see ref. 4):

$$H = \frac{J}{3}\sum_{\langle ij\rangle} S_i S_j + Da^3 \sum_{(ij)} \left[\frac{\hat{\mathbf{e}}_i \cdot \hat{\mathbf{e}}_j}{|\mathbf{r}_{ij}|^3} - \frac{3(\hat{\mathbf{e}}_i \cdot \mathbf{r}_{ij})(\hat{\mathbf{e}}_j \cdot \mathbf{r}_{ij})}{|\mathbf{r}_{ij}|^5} \right] S_i S_j \quad (1)$$

The distance between spins is r_{ij}, and $a \approx 3.54$ Å is the pyrochlore nearest-neighbour distance. $D = \mu_0 \mu^2/(4\pi a^3) = 1.41$ K is the coupling constant of the dipolar interaction.

Spin ice was identified as a very unusual magnet when it was noted that it does not order to the lowest temperatures T even though it appeared to have ferromagnetic interactions[3]. Indeed, spin ice was found to have a residual entropy at low T (ref. 5), which is well-approximated by the Pauling entropy for water ice, $S \approx S_P = (1/2)\log(3/2)$ per spin. Pauling's entropy measures the huge ground-state degeneracy arising from the so-called ice rules. In the context of spin ice, its observation implies a macroscopically degenerate ground state manifold obeying the 'ice rule' that two spins point into each vertex of the diamond lattice, and two out.

We contend that excitations above this ground-state manifold—that is, defects that locally violate the ice rule—are magnetic monopoles with the necessary long-distance properties. From the perspective of the seemingly local physics of the ice rule, the emergence of monopoles at first seems rather surprising. We will probe deeper into how the long-range magnetic interactions contained in equation (1) give rise to the ice rule in the first place. We then incorporate insights from recent progress in understanding the entropic physics of spin ice, and the physics of fractionalization in high dimensions[11–15], of which our monopoles will prove to be a classical instance.

We consider a modest deformation of equation (1), loosely inspired by Nagle's work[16] on the 'unit model' description of water ice: we replace the interaction energy of the magnetic dipoles living on pyrochlore sites with the interaction energy of dumbbells consisting of equal and opposite magnetic charges that live at the ends of the diamond bonds (see Fig. 2). The two ways of assigning charges on each diamond bond reproduce the two orientations of the original dipole. Demanding that the dipole moment of the spin be reproduced quantitatively fixes the value of the charge at $\pm\mu/a_d$, where the diamond lattice bond length $a_d = \sqrt{3/2}\, a$.

The energy of a configuration of dipoles is computed as the pairwise interaction energy of magnetic charges, given by the magnetic Coulomb law:

$$V(r_{\alpha\beta}) = \begin{cases} \frac{\mu_0}{4\pi}\frac{Q_\alpha Q_\beta}{r_{\alpha\beta}} & \alpha \neq \beta \\ \frac{1}{2}v_0 Q_\alpha^2 & \alpha = \beta \end{cases} \quad (2)$$

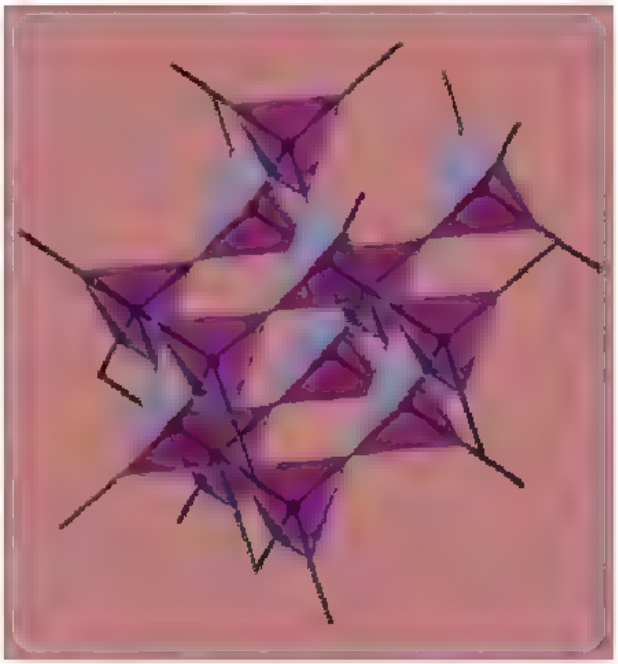


Figure 1 | The pyrochlore and diamond lattices. The magnetic moments in spin ice reside on the sites of the pyrochlore lattice, which consists of corner-sharing tetrahedra. These are at the same time the midpoints of the bonds of the diamond lattice (black) formed by the centres of the tetrahedra. The ratio of the lattice constant of the diamond and pyrochlore lattices is $a_d/a = \sqrt{3/2}$. The Ising axes are the local [111] directions, which point along the respective diamond lattice bonds

[1]Rudolf Peierls Centre for Theoretical Physics, Oxford University, Oxford OX1 3NP, UK. [2]Max-Planck-Institut für Physik komplexer Systeme, 01187 Dresden, Germany. [3]PCTP and Department of Physics, Princeton University, Princeton, New Jersey 08544, USA.

where Q_α denotes the total magnetic charge at site α in the diamond lattice, and $r_{\alpha\beta}$ is the distance between two sites. The finite 'self-energy' $v_0/2$ is required to reproduce the net nearest-neighbour interaction correctly. Equation (2)—which is derived in detail in the Supplementary Information—is equivalent to the dipolar energy equation (1), up to corrections that are small everywhere, and vanish with distance at least as fast as $1/r^5$.

We consider first the ground states of the system. The total energy is minimized if each diamond lattice site is net neutral, that is, we must orient the dumbbells so that $Q_\alpha = 0$ on each site. But this is just the above-mentioned ice rule, as illustrated in Fig. 2. Thus, one of the most remarkable features of spin ice follows directly from the dumbbell model: the measured low-T entropy agrees with the Pauling entropy (which follows from the short-distance ice rules), even though the dipolar interactions are long-range.

We now turn to the excited states. Naively, the most elementary excitation involves inverting a single dipole / dumbbell to generate a local net dipole moment 2μ. However, this is misleading in a crucial sense. The inverted dumbbell in fact corresponds to two adjacent sites with net magnetic charge $Q_\alpha = \pm q_m = \pm 2\mu/a_d$—a nearest-neighbour monopole–antimonopole pair. As shown in Fig. 2e, the monopoles can be separated from one another without further violations of local neutrality by flipping a chain of adjacent dumbbells. A pair of monopoles separated by a distance r experiences a Coulombic interaction, $-\mu_0 q_m^2/(4\pi r)$, mediated by monopolar magnetic fields, see Fig. 3.

This interaction is indeed magnetic, hence the presence of the vacuum permeability μ_0, and not $1/\varepsilon_0$, the inverse of the vacuum permittivity. It takes only a finite energy to separate the monopoles to infinity (that is, they are deconfined), and so they are the true elementary excitations of the system: the local dipolar excitation fractionalizes.

By taking the pictures from the dumbbell representation seriously, we may be thought somehow to be introducing monopoles where there were none to begin with. In general, it is of course well known that a string of dipoles arranged head to tail realizes a monopole–antimonopole pair at its ends[17]. However, to obtain deconfined monopoles, it is essential that the cost of creating such a string of dipoles remain bounded as its length grows, that is, the relevant string tension should vanish. This is evidently not true in a vacuum (such as that of the Universe) where the growth of the string can only come at the cost of creating additional dipoles. Magnetic materials, which come equipped with vacua (ground states) filled with magnetic dipoles, are more promising. However, even here a dipole string is not always a natural excitation, and when it is—for example, in an ordered ferromagnet – a string of inverted dipoles is accompanied by costly domain walls along its length (except, as usual, for one-dimensional systems[18]), causing the incipient monopoles to remain confined.

The unusual properties of spin ice arise from its exotic ground states. The ice rule can be viewed as requiring that two dipole strings enter and exit each site of the diamond lattice. In a typical spin-ice ground state, there is a 'soup' of such strings: many dipole strings of arbitrary size and shape can be identified that connect a given pair of sites. Inverting the dipoles along any one such string creates a monopole–antimonopole pair on the sites at its ends. The associated energy cost does not diverge with the length of the string, unlike in the case of an ordered ferromagnet, because no domain walls are created along the string, and the monopoles are thus deconfined.

We did not make reference to the Dirac condition[19] that the fundamental electric charge e and any magnetic charge q must exhibit the relationship $eq = nh/\mu_0$ whence any monopoles in our universe must be quantized in units of $q_D = h/\mu_0 e$. This follows from the monopole being attached to a Dirac string, which has to be unobservable[17]. By contrast, the string soup characteristic of spin ice at low temperature

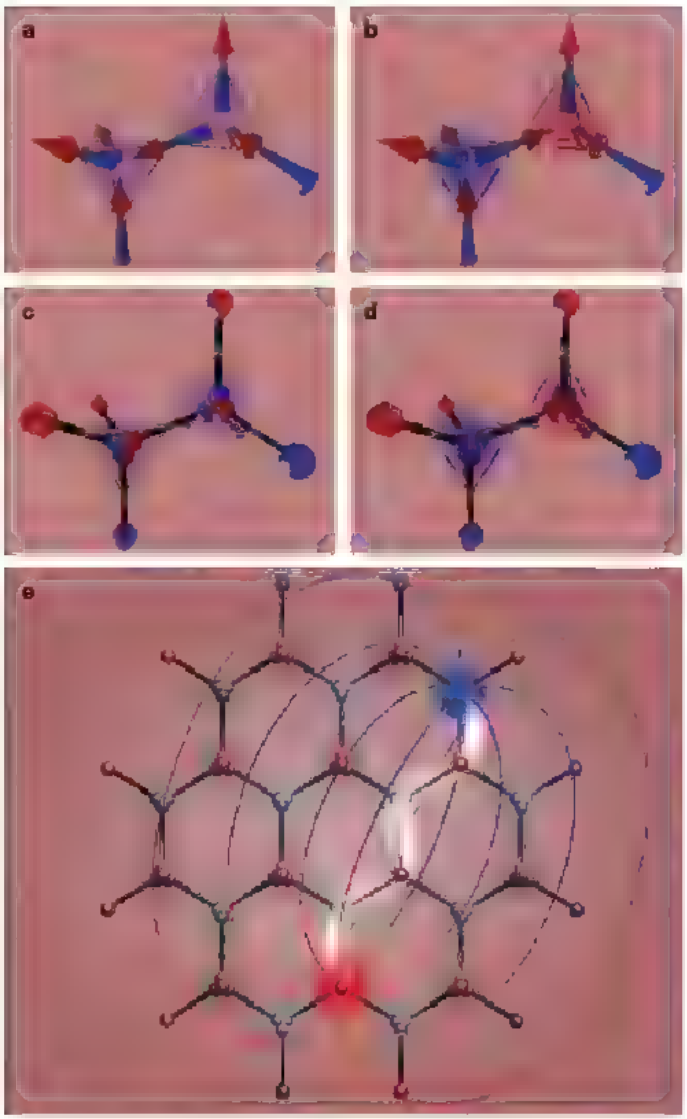


Figure 2 | Mapping from dipoles to dumbbells. The dumbbell picture (**c**, **d**) is obtained by replacing each spin in **a** and **b** by a pair of opposite magnetic charges placed on the adjacent sites of the diamond lattice. In the left panels (**a**, **c**), two neighbouring tetrahedra obey the ice rule, with two spins pointing in and two out, giving zero net charge on each site. In the right panels (**b**, **d**), inverting the shared spin generates a pair of magnetic monopoles (diamond sites with net magnetic charge). This configuration has a higher net magnetic moment and it is favoured by an applied magnetic field oriented upward (corresponding to a [111] direction). **e**, A pair of separated monopoles (large red and blue spheres). A chain of inverted dipoles ('Dirac string') between them is highlighted in white, and the magnetic field lines are sketched.

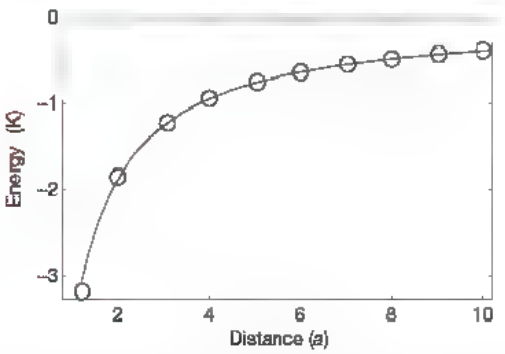


Figure 3 | Monopole interaction. Comparison of the magnetic Coulomb energy $-\mu_0 q_m^2/(4\pi r)$ (equation (2); solid line) with a direct numerical evaluation of the monopole interaction energy in dipolar spin ice (equation (1); open circles), for a given spin-ice configuration (Fig. 2e), as a function of monopole separation.

makes the strings energetically unimportant, although they are observable and are therefore not quantized.

Indeed, the monopoles in spin ice have a magnitude $q_m = 2\mu/a_d = 2(\mu/\mu_B)(\alpha\lambda_C/2\pi a_d)q_D \approx q_D/8{,}000$, where λ_C is the Compton wavelength for an electron, and α is the fine-structure constant. The charge of a monopole in spin ice can even be tuned continuously by applying pressure, because this changes the value of μ/a_d.

The monopoles are sources and sinks of the magnetic field **H**, as is appropriate to the condensed matter setting. More precisely, as in other instances of fractionalization[20], we can define a 'smeared' magnetic charge $\rho_m(\mathbf{R}) = \int d^3\mathbf{r}' \exp\left(-|\mathbf{r}'-\mathbf{R}|^2/\xi^2\right) \nabla\cdot\mathbf{H}$, where $\nabla\cdot\mathbf{H}$ is the divergence of the magnetic field. For a monopole at the origin, separated by $L \gg \xi \gg a$ from any other monopoles, this gives $\rho_m(0) = \pm q_m$. The form of the magnetic induction **B** is also monopolar but with the important difference that a compensating flux travels along the (unquantized) 'Dirac string' of flipped dipoles created along with the monopole (see Fig. 2).

Our magnetic monopoles would in principle show up in one of the best-known monopole searches, the Stanford experiment to detect fundamental magnetic monopoles from cosmic radiation. This experiment is based on the fact that a long-lived current is induced in a superconducting ring when a monopole passes through it[8]. We can easily check that the presence of the Dirac string of flipped dipoles is immaterial to the establishment of a current.

The above observations are the central qualitative results of our work: ice-rule-violating defects are deconfined monopoles of **H**, they exhibit a genuine magnetic Coulomb interaction (see equation (2)), and they produce Faraday electromotive forces in the same way as elementary monopoles would.

We re-emphasize that the ice rule alone does not permit a consistent treatment of the excited states of the physical problem: crucially, the energetic interaction between our defects is absent altogether. Also, in previous discussions of the purely ice-rule problem and related short-range problems[1–13] it has been noted that the defects do acquire a purely entropic Coulomb (that is, $1/r$) interaction, which has a strength that vanishes proportionally to T at low temperatures. This interaction will be present in addition to the magnetic Coulomb interaction discussed in this paper, and is clearly much smaller as $T \to 0$. Also, it will not be accompanied by a magnetic field, it will not renormalize the monopole charge, and it will not be felt by a stationary magnetic test particle that is embedded in the lattice but is not attached to a lattice site.

The most satisfactory way to demonstrate the presence of a monopole would be to measure the force on magnetic test particles, say by a Rutherford scattering experiment or by clever nanotechnological means. Unfortunately, given the lack of elementary magnetic monopoles, we would have to use dipoles as test particles, which significantly weakens such signatures.

An alternative strategy is to look for consequences of the presence of magnetic monopoles in the collective behaviour of spin ice. This is most elegantly achieved by applying a magnetic field in the [111] crystallographic direction. Such a field acts as a (staggered) chemical potential (see Fig. 2), favouring the creation of monopoles of a given sign on either sublattice of the diamond lattice.

We thus have a tuneable lattice gas of magnetic monopoles on the diamond lattice. The basic structure of the phase diagram as a function of magnetic field and temperature can be inferred from work by Fisher and collaborators[21] in the context of ionic lattice gases and Coulombic criticality. At high T, there is no phase transition but a continuous crossover between the high- and low-density phases as the chemical potential is varied. At low T, a first-order phase transition separates the two regimes. This transition terminates in a critical point at (h_c, T_c), not unlike the liquid–gas transition of water. This serves as a useful diagnostic, because the liquid–gas transition is absent for a nearest-neighbour spin-ice model, in which defects interact only entropically. In that case, it is known that there cannot be a first-order transition in the limit of low T (ref. 22).

To confirm this scenario, we have demonstrated by Monte Carlo simulations that the actual phase diagram of dipolar spin-ice model has precisely this structure. To rule out the appearance of the liquid–gas transition being due to effects introduced by the approximations leading to equation (2), we simulated directly the original dipolar spin-ice model, equation (1). The resulting phase diagram is depicted in Fig. 4. The critical endpoint is located around $(T_c, h_c) = (0.57 \pm 0.06\,\mathrm{K},\ 0.86 \pm 0.03\,\mathrm{T})$. The error bars are mainly due to finite-size effects, as the intensive nature of the simulations of long-range dipolar interactions prohibits simulating very large systems.

This scenario is indeed observed experimentally in spin-ice materials[6,7], and our results provide a natural explanation. Spin ice in a [111] magnetic field is a problem that has already attracted considerable attention. The low-density phase of monopoles is known as kagome ice, a quasi-two-dimensional phase with algebraic correlations and a reduced residual entropy[6,7,23]. The high-density phase is an ordered state with maximal polarization along the field direction. Experimental results on the liquid–gas transition and its endpoint are also displayed in Fig. 4 for comparison. Our numerical results are in good qualitative agreement with both experiment and the analytic calculations of ref. 21. Our value of the critical field agrees with ref. 6 to within a few per cent, which is less than the uncertainty due to demagnetization effects[6,7]. However, the experimental value of T_c is about a third lower than the numerical one, most probably due to farther-neighbour (exchange) interaction terms, which—although small—can shift the location of a transition temperature considerably[10].

The presence of a liquid–gas transition was noted to be very remarkable because there are few, if any, other experimentally known instances in localized spin systems[6]. No mechanism was known to account for this phenomenon, and our theory of magnetic monopoles fills this gap.

The existence of magnetic monopoles in a condensed matter system is exciting in itself. (The monopoles appearing in the interesting work on the anomalous Hall effect are not excitations and do not involve the physical magnetic field[24].) Moreover, these monopoles are a rare instance of high-dimensional fractionalization, of interest

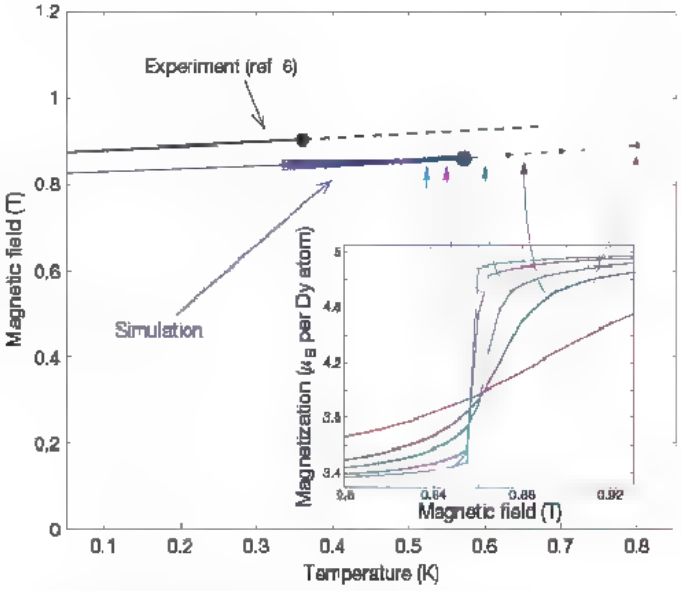


Figure 4 | Phase diagram of spin ice in a [111] field. The location of the monopole liquid–gas transition from numerics (blue line) compared to experiment (black line; ref. 6). The solid lines are first-order transitions terminated by critical endpoints (filled circles). The dashed lines are crossovers. The inset shows magnetization curves showing the onset of first-order behaviour as the temperature is lowered. Our simulations cover the range $0.335\,\mathrm{K} < T < 0.8\,\mathrm{K}$ for 1,024 spins. At the lowest temperatures, the parallel tempering code we use in our simulations of the Ewald-summed dipolar interaction no longer completely suppresses the hysteresis, and we have extended the first-order transition line using Clausius–Clapeyron.

in fields as diverse as correlated electrons and topological quantum computing[25]. We hope our analysis will encourage experiments aimed at directly detecting these monopoles. There are many avenues to explore in search of useful signatures, among them scattering, transport and noise measurements, and flux detection.

Received 27 June; accepted 29 October 2007.

1. Milton, K. A. Theoretical and experimental status of magnetic monopoles. *Rep. Prog. Phys.* 69, 1637–1712 (2006).
2. Laughlin, R. B. Nobel lecture: Fractional quantization. *Rev. Mod. Phys.* 71, 863–874 (1999).
3. Harris, M. J., Bramwell, S. T., McMorrow, D. F., Zeiske, T. & Godfrey, K. W. Geometrical frustration in the ferromagnetic pyrochlore $Ho_2Ti_2O_7$. *Phys. Rev. Lett.* 79, 2554–2557 (1997).
4. Bramwell, S. T. & Gingras, M. J. P. Spin ice state in frustrated magnetic pyrochlore materials. *Science* 294, 1495–1501 (2001).
5. Ramirez, A. P., Hayashi, A., Cava, R. J., Siddharthan, R. & Shastry, S. Zero-point entropy in 'spin ice'. *Nature* 399, 333–336 (1999).
6. Aoki, H., Sakakibara, T., Matsuhira, K. & Hiroi, Z. Magnetocaloric effect study on the pyrochlore spin ice compound $Dy_2Ti_2O_7$ in a [111] magnetic field. *J. Phys. Soc. Jpn* 73, 2851–2856 (2004).
7. Higashinaka, R., Fukazawa, H., Deguchi, K. & Maeno, Y. Low temperature specific heat of $Dy_2Ti_2O_7$ in the kagome ice state. *J. Phys. Soc. Jpn* 73, 2845–2850 (2004).
8. Cabrera, B. First results from a superconductive detector for moving magnetic monopoles. *Phys. Rev. Lett.* 48, 1378–1381 (1982).
9. Siddharthan, R. *et al.* Ising pyrochlore magnets: low-temperature properties, ice rules, and beyond. *Phys. Rev. Lett.* 83, 1854–1857 (1999).
10. Ruff, J. P. C., Melko, R. G. & Gingras, M. J. P. Finite-temperature transitions in dipolar spin ice in a large magnetic field. *Phys. Rev. Lett.* 95, 097202 (2005).
11. Huse, D. A., Krauth, W., Moessner, R. & Sondhi, S. L. Coulomb and liquid dimer models in three dimensions. *Phys. Rev. Lett.* 91, 167004 (2003).
12. Hermele, M., Fisher, M. P. A. & Balents, L. Pyrochlore photons: The U(1) spin liquid in a S=1/2 three-dimensional frustrated magnet. *Phys. Rev. B* 69, 064404 (2004).
13. Henley, C. L. Power-law spin correlations in pyrochlore antiferromagnets. *Phys. Rev. B* 71, 014424 (2005).
14. Fulde, P., Penc, K. & Shannon, N. Fractional charges in pyrochlore lattices. *Ann. Phys.* 11, 892–900 (2002).
15. Levin, M. & Wen, X. G. Quantum ether: Photons and electrons from a rotor model. *Phys. Rev. B* 73, 035122 (2006).
16. Nagle, J. F. Theory of the dielectric constant of ice. *Chem. Phys.* 43, 317–328 (1979).
17. Jackson, J. D. *Classical Electrodynamics* 251–260, Ch. 6.12–6.13 (Wiley, New York, 1975).
18. Saitoh, E., Miyajima, H., Yamaoka, T. & Tatara, G. Current-induced resonance and mass determination of a single magnetic domain wall. *Nature* 432, 203–206 (2004).
19. Dirac, P. A. M. Quantised singularities in the electromagnetic field. *Proc. R. Soc. A* 133, 60–72 (1931).
20. Rajaraman, R. Fractional charge. Preprint at (http://arxiv.org/abs/cond-mat/0103366v1) (2001).
21. Kobelev, V., Kolomeisky, A. B. & Fisher, M. E. Lattice models of ionic systems. *J. Chem. Phys.* 116, 7589–7598 (2002).
22. Heilmann, O. J. & Lieb, E. H. Theory of monomer-dimer systems. *Commun. Math. Phys.* 25, 190–232 (1972).
23. Isakov, S. V., Raman, K. S., Moessner, R. & Sondhi, S. L. Magnetization curve of spin ice in a [111] magnetic field. *Phys. Rev. B* 70, 104418 (2004).
24. Fang, Z. *et al.* The anomalous Hall effect and magnetic monopoles in momentum space. *Science* 302, 92–95 (2003).
25. Kitaev, A. Yu. Fault tolerant quantum computing. *Ann. Phys.* 303, 2–30 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank S. Bramwell, J. Chalker, C. Chamon and S. Kivelson (especially for pointing out ref. 8) for discussions. This work is supported in part by EPSRC (CC), and NSF (SLS). We also thank A. Canossa for support with the graphics.

Author Contributions All authors contributed equally to the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.C. (castel@physics.ox.ac.uk).

LETTERS

Three-dimensional atomic-scale structure of size-selected gold nanoclusters

Z. Y. Li[1], N. P. Young[1], M. Di Vece[1], S. Palomba[1], R. E. Palmer[1], A. L. Bleloch[3], B. C. Curley[2], R. L. Johnston[2], J. Jiang[4] & J. Yuan[4]

An unambiguous determination of the three-dimensional structure of nanoparticles is challenging[1]. Electron tomography requires a series of images taken for many different specimen orientations[2]. This approach is ideal for stable and stationary structures[3]. But ultrasmall nanoparticles are intrinsically structurally unstable and may interact with the incident electron beam[4–6], constraining the electron beam density that can be used and the duration of the observation. Here we use aberration-corrected scanning transmission electron microscopy[7], coupled with simple imaging simulation, to determine with atomic resolution the size, three-dimensional shape, orientation and atomic arrangement of size-selected gold nanoclusters that are preformed in the gas phase and soft-landed on an amorphous carbon substrate. The structures of gold nanoclusters containing 309±6 atoms can be identified with either Ino-decahedral, cuboctahedral or icosahedral geometries. Comparison with theoretical modelling of the system suggests that the structures are consistent with energetic considerations. The discovery that nanoscale gold particles function as active and selective catalysts for a variety of important chemical reactions has provoked much research interest in recent years[8–12]. We believe that the detailed structure information we provide will help to unravel the role of these nanoclusters in size- and structure-specific catalytic reactions[11,12]. We note that the technique will be of use in investigations of other supported ultrasmall metal cluster systems.

Scanning transmission electron microscopy (STEM), in the mode where incoherently scattered electrons are collected by a high-angle annular dark field (HAADF) detector, is appealing as a method of probing three-dimensional structure of nanoparticles (via an analysis of the intensity map from a single HAADF-STEM image) because its intensity is strongly dependent not only on the atomic number Z of the observed atoms but also on the number of atoms in a column[13,14]. The recent successful implementation of spherical aberration (C_s) correction in STEM[15] enables us to achieve the same analysis, but now at the atomic scale. We show that, by combining quantitative HAADF-STEM analysis with molecular-dynamics-based model structure search procedures and realistic image contrast simulations, it is possible to identify not only the size and shape but also the structure and orientation of soft-landed Au nanoclusters.

We demonstrate this for size-selected Au_N (where $N = 309 \pm 6$) clusters, where Au_{309} is known to be a possible 'magic number' nanocluster (see Supplementary Information). Figure 1a–c displays three high-resolution HAADF images taken from a Au_{309} nanoparticle. The images show outline shapes, which are approximately pentagonal, square and hexagonal, respectively. Close inspection of the intensity variation within the individual cluster images further reveals that the arrangement of the atomic columns varies from one cluster morphology to the other. Single Au atoms are also visible in the vicinity of the clusters or occasionally some distance away (two such atoms are marked by the circles in Fig. 1a and c). We have imaged the same clusters in several successive frames. The clusters sometimes move/rotate by small amounts and sometimes their structures change too. The individual atoms imaged on the carbon surface around the cluster also move from one frame to the next. The images shown in Fig. 1 are the first-pass images in each case.

To establish a foundation on which to analyse quantitatively the structure of the Au_{309} clusters, we carried out a series of integrated HAADF intensity measurements on size-selected Au clusters in the size range $N = 55$–$1{,}500$ atoms. For each sample, the HAADF intensity integrated over each cluster shows a narrow distribution (see Fig. 2 inset for $N = 309$). The mean intensity value taken for each cluster size is plotted as a function of the selected-size N in Fig. 2, where the standard deviation is used for estimating the error bars. The finite width of the distribution can be attributed partly to the

Figure 1 | High-resolution HAADF-STEM images of Au_{309} clusters on a carbon film. Typical images show various outline shapes, that is, cluster projections: pentagon (**a**), square (**b**) and hexagon (**c**). The intensity variation within the clusters clearly demonstrates atomic-column resolution. Single atoms can be seen in the vicinity of the clusters, as indicated by the circle in **c**, and occasionally some distance away, as indicated by the circle in **a**. Resolution of the mass selector is ±2%.

[1]Nanoscale Physics Research Laboratory, School of Physics and Astronomy, [2]School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK. [3]UK SuperSTEM Laboratory, Daresbury Laboratory, Daresbury WA4 4AD, UK. [4]Beijing Electron Microscopy Centre, Laboratory of Advanced Materials and Department of Materials Science and Engineering, Tsinghua University, Beijing 100084, China.

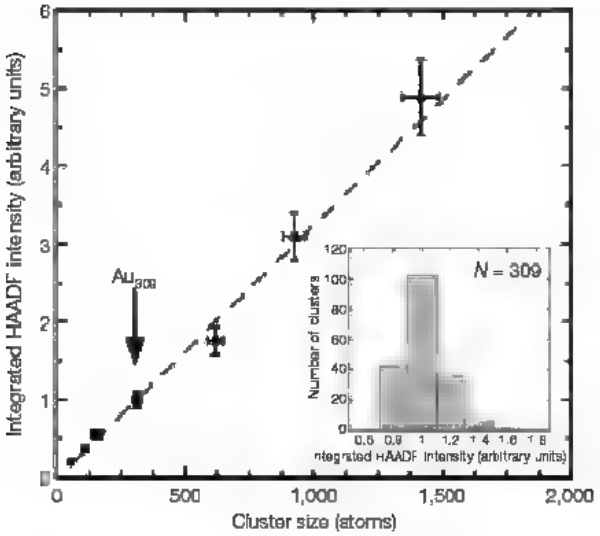


Figure 2 | Relationship between integrated HAADF intensity and size of gold clusters. Integrated HAADF intensity of size-selected Au clusters on amorphous carbon film plotted as a function of the number of atoms they contain, showing a linear relationship. The line is drawn as a guide to the eye. Each data point is obtained from a statistical intensity distribution analysis over a large number of clusters with a given number of atoms. The standard deviation is used for estimating the error bars. An example of such a distribution for Au_{309} is shown in the inset.

resolution of the mass-selector (±2%) and suggests that clusters soft-landing on the surface do not suffer significant fragmentation or coalescence. This is consistent with the results of our detailed image analysis, which reveals no extensive rafts of single atom layers on the amorphous carbon support, apart from a few 'shake-off' atoms. Figure 2 shows that the integrated HAADF intensity from the clusters increases linearly with the number of constituent atoms up to about $N = 1{,}500$. This linearity implies that, at small cluster size, atoms within the cluster contribute equally to the total scattered electron signal detected by the HAADF detector.

The linearity shown in Fig. 2 also suggests that the HAADF intensities for the individual atomic columns in Fig. 1 can be directly associated with the number of atoms in each column. The clear five-fold symmetry in the atomic column arrangement in Fig. 1a, for example, suggests that the cluster has Ino-decahedral geometry and is oriented on the substrate such that the five-fold axis is parallel to the electron beam, as shown in the hard-sphere representation in Fig. 3a. Figure 3b displays an illustrative line intensity profile from the centre of this cluster to one of the corners, averaging over three experimental pixels (equal to 0.9 Å). Five peaks and a shoulder (marked by the arrow) are apparent, with the peak intensity decreasing gradually towards the corner. Using a simple kinematical approach, the simulated HAADF-STEM image of the decahedral Au_{309} cluster is shown in Fig. 3c, together with the intensity profile (the solid red curve). The correspondence between the simulated profile (Fig. 3c), and the experimental profile (Fig. 3b), with respect to both the peak positions and the relative peak intensities, is remarkable, indicating the correct identification of the atomic column structure. An icosahedron also has a five-fold rotational symmetry axis; however, the rotation-reflection symmetry of this structure results in a STEM image having ten-fold symmetry, and the technique described has the potential to discriminate between these two possible structures (see Supplementary Information). We have also conducted a full dynamical calculation using the multislice method[15]. The corresponding line profile is shown by the dashed line in Fig. 3c. The similarity between the two simulated line profiles confirms the validity of the simple kinematical approximation for HAADF-STEM image simulation of the Au_{309} clusters and that the quantization of the HAADF intensity correlates directly with the quantization of the number of the atoms.

Close comparison between Fig. 3b and c also highlights a discrepancy in the atom columns at the edge of the cluster. The experimental intensity of the outermost atomic column is significantly lower than those predicted by either simulation. In addition, an extra shoulder appears in the experimental profile, as indicated by the arrow, with a peak intensity lower than that of the isolated single Au atom observed on the same sample (Fig. 1a). This shoulder exists for all the clusters inspected, irrespective of their shape. The discrepancy cannot be wholly explained by effects such as the rocking movement of the cluster under the electron beam, electron-beam scan instability or noise from other sources, because all these effects would result in the smearing out of the overall image. We take it as evidence that atoms in the surface layer of the clusters fluctuate significantly, on a timescale shorter than the period for the data acquisition. This is similar

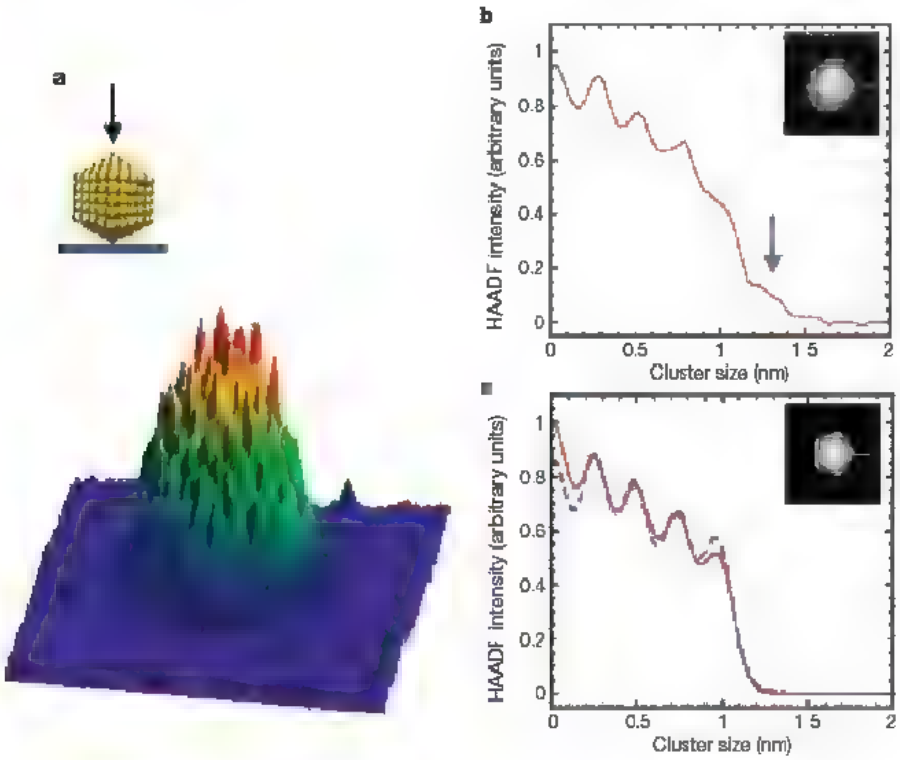


Figure 3 | Three-dimensional atomic structure of a gold cluster ($N = 309 \pm 6$). **a**, Three-dimensional atom density profile of Au_{309}, derived from Fig. 1a. A hard-sphere model for an Ino-decahedral structure is shown with the electron beam (arrow) parallel to the five-fold axis. **b**, Experimental intensity line profile taken from the central atom column of the cluster to one of the corners (indicated in inset with red line. **c**, Simulated HAADF-STEM image (inset) obtained with a simple kinematical approach, of an Au_{309} cluster with Ino-decahedral geometry. An intensity profile (solid curve) across one ridge (indicated in inset with red line) is compared with the result from a full dynamical multislice calculation (dashed line).

to the dynamic motion of surface atoms previously observed for larger nanoparticles[17,18].

The observed structures of the Au clusters can be understood from their calculated total potential energies for different polyhedral geometries (icosahedral, Ino-decahedral and cuboctahedral) as a function of the number of constituent atoms. After local energy minimization, it was found that, for very small Au clusters ($N < 100$), the icosahedral structure is much more stable than the Ino-decahedral and the cuboctahedral structures. The total potential energy is in the order of: icosahedral < Ino-decahedral < cuboctahedral. However, for the larger clusters ($N \approx 500$–$1{,}000$), the order of stability begins to change, with the Ino-decahedral structure becoming more stable than the icosahedral geometry: Ino-decahedral < icosahedral < cuboctahedral. Further increasing the cluster size results in the order of stability changing to: Ino-decahedral < cuboctahedral < icosahedral. For Au_{309}, the difference in total energy between different geometries was less than 1.2 eV (that is, less than 3.88 meV per atom) from the most stable to the least stable. Moreover, there are many local energy minima and the energy barriers between these structures are small. These results support our experimental findings that no one structure dominates. For Au_{309}, we see a similar proportion of clusters with Ino-decahedral (32%) and cuboctahedral (25%) structures and a much lower population of icosahedral structures (8%). In the remaining population, some clusters show irregular facets and some do not show any ordered geometry, possibly because of significant rearrangement of the outer-shell atoms, akin to the solid–liquid phase coexistence predicted for other systems[19]. Given the narrow size distribution of the deposited clusters, our results may shed light on the relative structural stabilities of the various cluster isomers in the gas phase, information that has solicited many theoretical investigations but little hard experimental evidence.

In conclusion, we have demonstrated the suitability of high-angle annular dark-field imaging in the aberration-corrected STEM for detailed structural and stability analysis of size-selected metallic clusters on solid supports at atomic resolution. The multiplicity of cluster geometries revealed by our detailed study of the atomic arrangement of soft-landed Au_{309} clusters on amorphous carbon supports is consistent with many local energy minima predicted for clusters of this size by cluster simulations. Evidence for increased fluctuations and motion of cluster surface atoms relative to the core atoms within the Au_{309} clusters may reflect an inherent property of the nanometre-sized gold clusters that could be related to their enhanced catalytic properties through much reduced coordination. Vertical depth information can be extracted from a single projection, with single-atom sensitivity, opening up the possibility to use the technique as a routine three-dimensional structural characterization tool for small nanoparticles at the atomic-scale level, with the help of image simulation based on *ab initio* cluster modelling including dynamical effects. The experimental approach has practical advantages, such as the more relaxed constraints on cluster stability[17,18]. When combined with time-lapsed imaging techniques, our approach could provide the dynamical insight into the atomistic structural changes of nanoparticles that commonly occur in some catalytic reactions[20].

METHODS SUMMARY

The gold clusters are formed by gas-phase condensation of sputtered atoms in a rare-gas atmosphere[21], size-selected by a lateral time-of-flight mass spectrometer[22] and soft-landed on an amorphous carbon support[23] for examination by high-angle annular dark-field STEM, using a spherical-aberration-corrected machine for the atomically resolved imaging[24]. The three-dimensional atomic structures of the size-selected clusters are obtained by comparison of the experimental results with both kinematic and dynamical image simulations[16,25], based on structural models optimized by a realistic many-body potential[26,27].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 22 June; accepted 13 November 2007.
Published online 9 December 2007.

1. Koga, K., Ikeshoji, T. & Sugawara, K. Size- and temperature-dependent structural transitions in gold nanoparticles. *Phys. Rev. Lett.* 92, 115504–115507 (2004)
2. Midgley, P. A. & Weyland, M. 3D electron microscopy in the physical sciences: the development of *Z*-contrast and EFTEM tomography. *Ultramicroscopy* 96, 413–431 (2003)
3. Arslan, I., Yates, T. J. V., Browning, N. D. & Midgley, P. A. Embedded nanostructures revealed in three dimensions. *Science* 309, 2195–2198 (2005).
4. Iijima, S. & Ichihashi, T. Structural instability of ultrafine particles of metals. *Phys. Rev. Lett.* 56, 616–619 (1986)
5. Ajayan, P. M. & Marks, L. D. Experimental evidence for quasimelting in small particles. *Phys. Rev. Lett.* 63, 279–282 (1989)
6. Marks, L. D. Experimental studies of small particle structures. *Rep. Prog. Phys.* 57, 603–649 (1994).
7. Batson, P. E., Dellby, N. & Krivanek, O. L. Sub-angstrom resolution using aberration corrected electron optics. *Nature* 418, 617–620 (2002)
8. Haruta, M. Size- and support-dependency in the catalysis of gold. *Catal. Today* 36, 153–166 (1997)
9. Sanchez, A. *et al.* When gold is not noble: nanoscale gold catalysts. *J. Phys. Chem. A* 103, 9573–9578 (1999)
10. Hughes, M. D. *et al.* Tunable gold catalysts for selective hydrocarbon oxidation under mild conditions. *Nature* 437, 1132–1135 (2005).
11. Tsunoyama, H., Sakurai, H., Negishi, Y. & Tsukuda, T. Size-specific catalytic activity of polymer-stabilized gold nanoclusters for aerobic alcohol oxidation in water. *J. Am. Chem. Soc.* 127, 9374–9375 (2005).
12. Chen, M. S. & Goodman, D. W. Structure-activity relationships in supported Au catalysts. *Catal. Today* 111, 22–33 (2006)
13. Li, Z. Y., Yuan, J., Chen, Y., Palmer, R. E. & Wilcoxon, J. P. Direct imaging of core-shell structure in silver-gold bimetallic nanoparticles. *Appl. Phys. Lett.* 87, 243103 (2005)
14. Li, Z. Y., Yuan, J., Chen, Y., Palmer, R. E. & Wilcoxon, J. P. Local three-dimensional visualization of nanoparticle assemblies. *Adv. Mater.* 17, 2885–2888 (2005).
15. Krivanek, O. L., Delby, N. & Lupini, A. R. Towards sub-angstrom electron beams. *Ultramicroscopy* 78, 1–11 (1999)
16. Kirkland, E. J. *Advanced Computing in Electron Microscopy* (Plenum Press, New York/London, 1998)
17. Smith, D. J., Petford-Long, A. K., Wallenberg, L. R. & Bovin, J.-O. Dynamic atomic-level rearrangements in small gold particles. *Science* 233, 872–875 (1986)
18. Bovin, J.-O., Wallenberg, R. & Smith, D. J. Imaging of atomic clouds outside the surfaces of gold crystals by electron microscopy. *Nature* 317, 47–49 (1985)
19. Schebarchov, D. & Hendy, S. C. Transition from icosahedral to decahedral structure in a coexisting solid-liquid nickel cluster. *Phys. Rev. Lett.* 95, 116101–116104 (2005)
20. Hansen, P. L. *et al.* Atom-resolved imaging of dynamic shape changes in supported copper nanocrystals. *Science* 295, 2053–2055 (2003).
21. Pratontep, S., Carroll, S. J., Xirouchaki, C., Streun, M. & Palmer, R. E. Size-selected cluster beam source based on radio frequency magnetron plasma sputtering and gas condensation. *Rev. Sci. Instrum.* 76, 045103–045109 (2005).
22. von Issendorff, B. & Palmer, R. E. A new high transmission infinite range mass selector for cluster and nanoparticle beams. *Rev. Sci. Instrum.* 70, 4497–4501 (1999)
23. Palmer, R. E., Pratontep, S. & Boyen, H.-G. Nanostructured surfaces from size-selected clusters. *Nature Mater.* 2, 443–448 (2003).
24. Bleloch, A. L. & Lupini, A. maging at the picoscale. *Mater. Today* 7, 42–48 (2004).
25. Jiang, J., Yuan, J. & Bleloch, A. L. Cluster-scale composition determination in a boron-rich compound. *Appl. Phys. Lett.* 91, 113107 (2007)
26. Clerc, F. & Rosato, V. Tight-binding potentials for transition metals and alloys. *Phys. Rev. B* 48, 22–33 (1993)
27. Johnston, R. L. Evolving better nanoparticles: Genetic algorithms for optimising cluster geometries. *Dalton Trans.* 4193–4207 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank Y. Chen for assistance with the electron microscopy work in Birmingham. We gratefully acknowledge the UK Engineering and Physical Science Research Council (EPSRC) and the EU for their financial support of the cluster work. The EPSRC funded the UK SuperSTEM facility at Daresbury Laboratory. N.P.Y. and B.C.C. acknowledge the EPSRC and the University of Birmingham for PhD funding, respectively. The work at Tsinghua University was supported by the Ministry of Science and Technology and Ministry of Education in China.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to Z.Y.L. (ziyouli@nprl.ph.bham.ac.uk).

LETTERS

Net carbon dioxide losses of northern ecosystems in response to autumn warming

Shilong Piao[1], Philippe Ciais[1], Pierre Friedlingstein[1], Philippe Peylin[2], Markus Reichstein[3], Sebastiaan Luyssaert[4], Hank Margolis[5], Jingyun Fang[6], Alan Barr[7], Anping Chen[8], Achim Grelle[9], David Y. Hollinger[10], Tuomas Laurila[11], Anders Lindroth[12], Andrew D. Richardson[13] & Timo Vesala[14]

The carbon balance of terrestrial ecosystems is particularly sensitive to climatic changes in autumn and spring[1–4], with spring and autumn temperatures over northern latitudes having risen by about 1.1 °C and 0.8 °C, respectively, over the past two decades[5]. A simultaneous greening trend has also been observed, characterized by a longer growing season and greater photosynthetic activity[6,7]. These observations have led to speculation that spring and autumn warming could enhance carbon sequestration and extend the period of net carbon uptake in the future[8]. Here we analyse interannual variations in atmospheric carbon dioxide concentration data and ecosystem carbon dioxide fluxes. We find that atmospheric records from the past 20 years show a trend towards an earlier autumn-to-winter carbon dioxide build-up, suggesting a shorter net carbon uptake period. This trend cannot be explained by changes in atmospheric transport alone and, together with the ecosystem flux data, suggest increasing carbon losses in autumn. We use a process-based terrestrial biosphere model and satellite vegetation greenness index observations to investigate further the observed seasonal response of northern ecosystems to autumnal warming. We find that both photosynthesis and respiration increase during autumn warming, but the increase in respiration is greater. In contrast, warming increases photosynthesis more than respiration in spring. Our simulations and observations indicate that northern terrestrial ecosystems may currently lose carbon dioxide in response to autumn warming, with a sensitivity of about 0.2 PgC °C^{-1}, offsetting 90% of the increased carbon dioxide uptake during spring. If future autumn warming occurs at a faster rate than in spring, the ability of northern ecosystems to sequester carbon may be diminished earlier than previously suggested[9,10].

The carbon balance of terrestrial ecosystems is highly sensitive to climate changes at the edges of the growing season[1–4]. In response to warmer springs, for example, several field studies have shown that boreal forests absorb more carbon[11,12] as a result of an earlier beginning of the growing season[13,14]. A strong autumn warming is currently occurring in eastern Asia and eastern North America[15]. However, little attention has been given to the impacts of this forcing on the terrestrial carbon cycle. We have analysed how interannual variations and trends in autumn temperatures have recently affected atmospheric CO_2 concentrations, ecosystem CO_2 fluxes measured by eddy covariance, and remotely sensed vegetation greenness values. A process-oriented terrestrial biosphere model (ORCHIDEE)[16] is combined with an atmospheric transport model (LMDZt)[17] to quantify the processes through which autumn warming controls the carbon balance of ecosystems (see Methods).

The seasonal cycle of atmospheric CO_2 concentrations provides an integrated measure of the net land–atmosphere carbon exchange (net ecosystem productivity; NEP) and its temporal characteristics[18,19]. We analysed the ten atmospheric CO_2 measurement records from the NOAA–ESRL air-sampling network[20], which cover at least 15 years of data in the Northern Hemisphere (Fig. 1 and Supplementary Table 1). The upward zero-crossing date of CO_2 was determined as the day when the de-trended atmospheric CO_2 seasonal cycle crosses the zero line from negative to positive values (see Methods). This date occurs in autumn at northern high-latitude stations and in early winter at northern tropical stations (Supplementary Table 1). We found that variations in the CO_2 zero-crossing date are negatively correlated with anomalies in autumn air temperatures[5] over a broad region surrounding each station by ±20° of latitude. All CO_2 records show a negative correlation, with four out of ten sites having statistically significant correlations (Supplementary Table 2). The probability that this occurs purely by chance is estimated to be about 10^{-5} if all station records are assumed to be independent (see Supplementary Information). The striking anti-correlation between autumnal temperature and CO_2 zero-crossing date is illustrated in Fig. 1a for the 23-year-long atmospheric measurement record of Point Barrow in northern Alaska ($R = -0.61$, $P = 0.002$). In contrast with the widespread influence of temperature, the upward CO_2 zero-crossing date shows no significant correlation with precipitation anomalies (Supplementary Table 2). If soil moisture calculated by the ORCHIDEE model (see Methods) is used instead of precipitation as a predictor of CO_2 upward zero-crossing dates, then only six of the ten sites show a positive correlation, and only three of the ten sites show a higher correlation with soil moisture than with temperature. Similar results are also inferred from a partial correlation analysis in which the controlling effects of other variables on temperature were removed (Supplementary Table 2).

We verified that the strong negative correlation between upward CO_2 zero-crossing date and temperature predominantly reflects climate-driven fluctuations in NEP, rather than interannual fluctuations in atmospheric transport. To do so, we prescribed either variable NEP or climatological NEP fluxes from ORCHIDEE to the

[1]LSCE, UMR CEA-CNRS Bâtiment 709, CE, L'Orme des Merisiers, F-91191 Gif-sur-Yvette, France. [2]Laboratoire de Biogéochimie Isotopique, LBI, Bâtiment EGER, F-78026 Thiverval-Grignon, France. [3]Max Planck Institute for Biogeochemistry, PO Box 100164, 07701 Jena, Germany. [4]Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium. [5]Faculté de foresterie et de géomatique, Université Laval, Sainte-Foy, Quebec G1K 7P4, Canada. [6]Department of Ecology, Peking University, Beijing 100871, China. [7]Climate Research Division, Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan S7N 3H5, Canada. [8]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA. [9]Department of Ecology, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden. [10]USDA Forest Service Northern Research Station, 271 Mast Road, Durham, New Hampshire 03824, USA. [11]Finnish Meteorological Institute, FIN-00101 Helsinki, Finland. [12]Department of Physical Geography and Ecosystems Analysis, Lund University, SE-22362 Lund, Sweden. [13]Complex Systems Research Center, University of New Hampshire, Durham, New Hampshire 03824, USA. [14]Department of Physical Sciences, University of Helsinki, PO Box 64, FIN-00014 Helsinki, Finland.

global transport model LMDZt driven by variable wind fields (see Methods). With the exception of the Mt Cimone (CMN) and Cape Kumukahi (KUM) stations, we found that the fluctuations in upward zero-crossing dates are driven mainly by changes in NEP, and only partly by interannual wind changes (see Methods, Supplementary Table 3 and Supplementary Fig. 1). We also verified that accounting for increasing ocean uptake and fossil fuel emissions in the LMDZt transport model did not significantly affect the zero-crossing dates because these two fluxes contribute less than 4% of the variation for all sites (except for station KUM). The possible changes in seasonal fossil fuel emissions over time may only marginally impact the upward CO_2 zero-crossing date changes (see Methods)

There is also a long-term trend in the autumn upward zero-crossing date of atmospheric CO_2 superimposed on interannual fluctuations. At Point Barrow, for instance, we determined a systematic advance of -0.40 day yr^{-1} (Fig. 1b), which was not primarily caused by changes in atmospheric transport, because the trends in zero-crossing date simulated with climatological ORCHIDEE fluxes and interannual transport are only about -0.12 day yr^{-1}. Overall, eight of ten sites show an earlier trend in upward zero-crossing date, with four sites being statistically significant (Fig. 1b). This trend towards earlier or increased ecosystem losses of CO_2 in autumn becomes

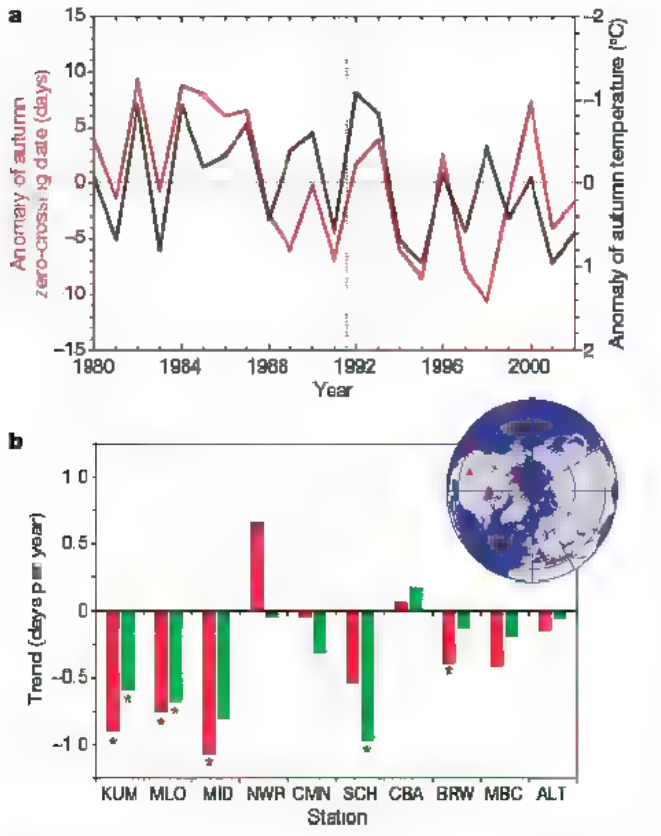


Figure 1 | Atmospheric CO_2 concentration data analysis from long-term records of the global NOAA-ERSL air-sampling network. **a**, Interannual variability in anomaly of upward zero-crossing date (red) observed at Point Barrow, Alaska, and the corresponding autumn (September to November) temperature (black) over the region between 51° and 90° N over the past two decades. Upward zero-crossing date is strongly anti-correlated with autumn temperature (slope = -5.4 days °C^{-1}; $R = -0.61$, $P = 0.002$). The vertical dotted line indicates the time of the eruption of Mount Pinatubo. **b**, Trends in upward zero-crossing date (red) and length of the net CUP (green) from long-term Northern Hemisphere atmospheric observations during at least the past 15 years (see Methods). The differences in the trends between autumn upward zero-crossing date and CUP reflects changes in the spring downward zero crossing. As a result of the earlier autumn upward zero-crossing date, CUP has persistently decreased by an average of 0.36 ± 0.38 days per year since 1980. The inset shows the distribution of the stations used in this study. Station abbreviations are defined in Supplementary Table 1.

apparent when analysing CO_2 data from the past decade, whereas it was non-existent in the CO_2 data from 1970 to 1994 (ref. 18) as a result of the time-frame of their analysis. This trend towards larger autumn CO_2 losses is not a legacy from drier summers[21], because atmospheric CO_2 data show that weaker summer CO_2 minima are not significantly associated with an advanced upward zero-crossing date at all sites. The advance in autumnal atmospheric CO_2 zero-crossing date clearly exceeds that of the spring zero-crossing date (Supplementary Table 3). Thus, the duration of the net carbon uptake period (CUP), defined as the difference between autumn upward and spring downward CO_2 zero-crossing dates, has on average decreased at nearly all Northern Hemisphere atmospheric CO_2 stations (Fig. 1b).

Next, we analysed 108 site-years of eddy-covariance CO_2 measurement data from 24 northern ecosystem sites (Supplementary Table 4) to quantify the response of the CUP ending date to interannual variations in autumn temperature (see Methods). All sites combined show that the CUP terminates systematically earlier when autumn conditions are warmer, and vice versa (Fig. 2). Further, stronger temperature anomalies seem to have stronger effects on ecosystem carbon balance than weak anomalies ($P < 0.05$). Hence, despite a large scatter in the individual yearly eddy-covariance CUP dates (see insets to Fig. 2), these micrometeorological observations corroborate the atmospheric concentration records.

The large-scale atmospheric concentration records, taken together with the ecosystem-scale eddy-covariance flux measurements (about 1 km^2) suggest that warmer temperatures in autumn increase ecosystem CO_2 losses by shortening the net CUP. This finding stands in apparent contradiction of the autumn 'greening' and longer-lasting vegetation activity detected at mid-to-high northern latitudes by remote sensing[6,7] and by numerous *in situ* phenological indicators[13,22]. However, the underlying mechanisms and processes are yet to be explained. NEP results from the balance between gross primary photosynthesis (GPP) and total ecosystem respiration (TER), necessitating separate investigations into the response of each gross flux to temperature changes. We provide some indication of possible controlling mechanisms by using the ORCHIDEE terrestrial biosphere simulation model forced by variable climate fields over the period 1980–2002 (see Methods). The model's ability to capture the timing of the CUP and the length of the growing season successfully was verified by using the following: first, eddy-covariance CO_2 flux measurements[16,23], second, satellite-derived observations of global leaf area index[24], and third, interannual and seasonal variations in atmospheric CO_2 (see Methods and Supplementary Fig. 1). Results from these studies suggest that it is possible to use this model tool to help in disentangling the response of photosynthesis, respiration and NEP to climate variability.

Simulated September to November NEP shows a trend towards increasing carbon losses in the Northern Hemisphere (north of 25° N) at a rate of 13 Tg C yr^{-1} ($P = 0.01$) during 1980–2002. In the ORCHIDEE model long-term simulation, the increasing autumn source of carbon to the atmosphere offsets about 90% of the increasing carbon sink in spring. This result is consistent with the atmospheric concentration analysis (Supplementary Table 3). We attribute the trend in net carbon loss during autumn to increases in TER (21 Tg C yr^{-1}) dominating over increasing GPP (8 Tg C yr^{-1} owing to delayed leaf senescence). In autumn, both modelled GPP and TER increase with increasing temperature, but the temperature sensitivity of TER (5.0 g C m^{-2} °C^{-1}) exceeds that of GPP (2.5 g C m^{-2} °C^{-1}). This is due to limitations of radiation and temperature on GPP during the autumn[24], and to soil desiccation carried over from the summer dry period[21]. As a result, autumn NEP is simulated to be an increasing source of CO_2 in response to warming, with a mean sensitivity of -2.5 g C m^{-2} °C^{-1} (or about -0.2 Pg C °C^{-1} north of 25° N), which is comparable to that derived from eddy-covariance measurements (-3.2 g C m^{-2} °C^{-1}; Fig. 2).

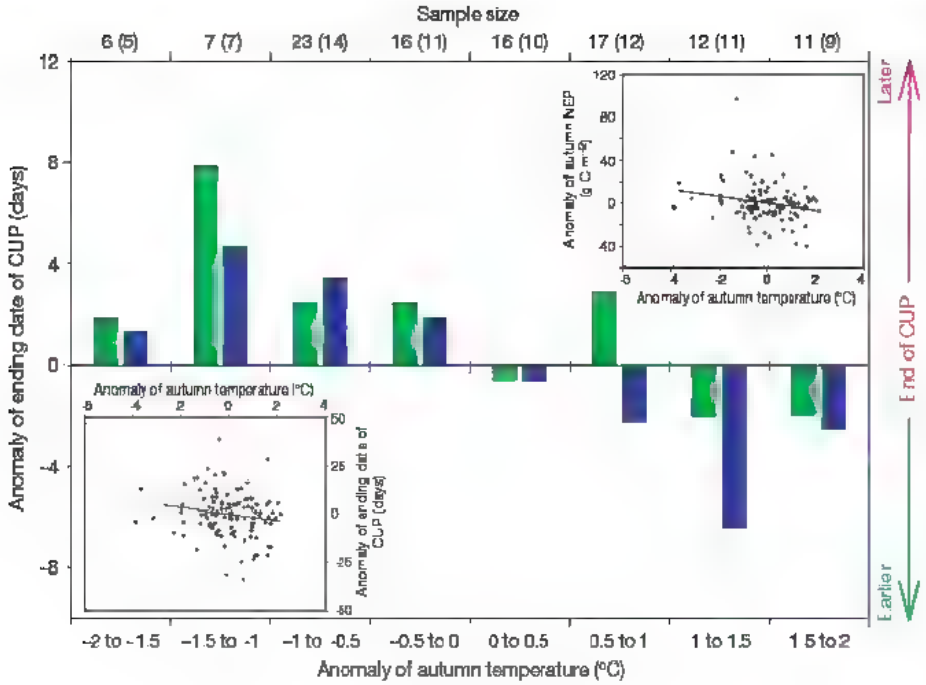


Figure 2 | Eddy-covariance flux data analysis from boreal sites in North America and Eurasia. A total of 108 site-years have been aggregated in this figure. The average (blue) and median (green) anomaly of ending date of net CUP is shown for different autumn temperature anomalies binned into 0.5 °C intervals. The top horizontal axis labels correspond to the number of site-years and sites (in parenthesis) in each temperature bin. The bottom left inset shows the relationships between ending date of CUP and temperature anomalies. There is a marginally negative correlation between autumn CUP ending date and temperature anomalies ($y = -1.7x$ 0.0087, $P = 0.07$). If we exclude the four site-years with the most extreme cold anomalies ($\Delta T < -2$ °C), the negative correlation between CUP ending date and temperature becomes highly significant ($P = 0.03$) and the slope is steeper ($y =$ 2.4x + 0.3007), suggesting that below a certain threshold of cold anomaly there is no further decrease in respiration. The top right inset shows the relationships between autumn NEP and temperature anomalies. A positive NEP value indicates an increased carbon uptake. Autumn was defined as the 60-day interval around the average CUP ending date for each site. Eddy-covariance data show increased carbon losses under warmer conditions, with a temperature sensitivity of NEP of 3.2 g C m^{-2} °C^{-1} ($y =$ 3.17x 5×10^{-6}, $P = 0.04$)

Our results suggest that net carbon uptake of northern ecosystems is being decreased in response to autumnal warming. The spatial distribution of the response of carbon flux to temperature, as projected by the ORCHIDEE model, is shown in Fig. 3. Warmer autumns coincide with greater than normal GPP (Fig. 3a). However, because of a concurrent stimulation of plant respiration, the geographical area where autumn NPP increases with temperature (slope > 5 g C m^{-2} °C^{-1}) is much less extensive than the area where GPP increases (Fig. 3b). The spatial pattern of the autumn increase in NPP in response to warming is remarkably similar to that of the NOAA/AVHRR vegetation index (NDVI) data[25] (Fig. 3d), suggesting that results from the ORCHIDEE model for NPP are realistic. However, this 'extra' autumn NPP is being accompanied by even more respiration in response to warming, so that the modelled NEP response shows systematic anomalous carbon losses during warmer autumns, in particular over North America and Europe (Fig. 3c).

Observed historical climate data[5] reveal that Eurasia experienced a stronger warming in spring (0.06 °C yr^{-1}, $P = 0.001$) than in autumn (0.02 °C yr^{-1}, $P = 0.15$) over the past two decades. In contrast, North America has experienced a larger warming in autumn (0.05 °C yr^{-1}, $P = 0.03$) than in spring (0.02 °C yr^{-1}, $P = 0.36$). In addition, a more significant and coherent greening pattern in Eurasia than in North America has been detected in the remote sensing data[7]. This suggests that the processes and the magnitude of seasonal changes in NEP in Eurasia and North America are different, which may control the annual carbon balance of their ecosystems. Further constraints on the spatial and temporal patterns of large-scale ecosystem fluxes will be delivered in the future from atmospheric inversions constrained with longer-term ecosystem flux data.

Applying the future Northern Hemisphere warming of 3.8–6.6 °C predicted by a climate model[26] to the sensitivity of the autumn zero-crossing date of atmospheric CO_2 at Point Barrow (about 5 days °C^{-1}) gives a projected advance of 19–33 days by the end of the twenty-first century. Previous model assessments of the response of land ecosystems to climate change concluded that terrestrial carbon sinks should peak by about the year 2050 and then diminish towards the end of the twenty-first century[9,10]. The asymmetrical

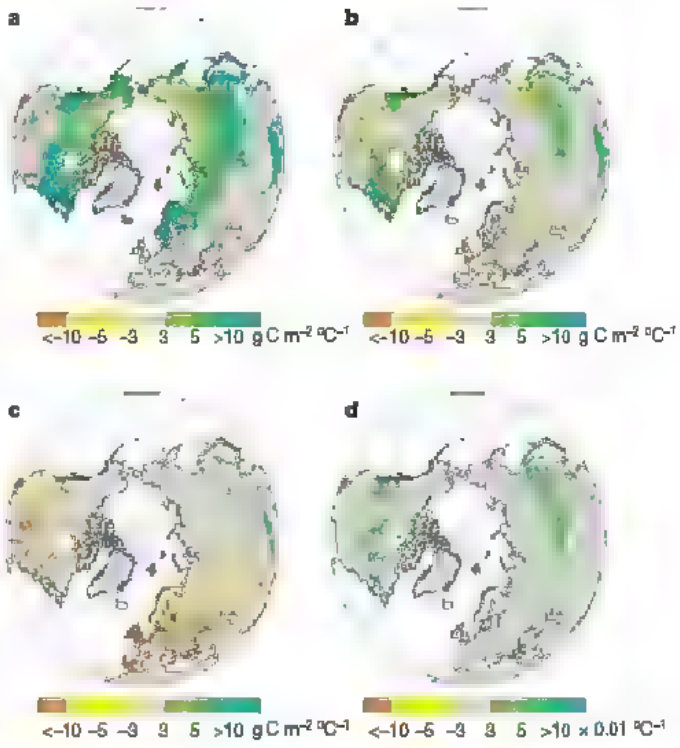


Figure 3 | A model view of the spatial distribution of the effects of autumn (September to November) temperature warming on gross and net carbon fluxes, obtained with the ORCHIDEE model. **a**, ORCHIDEE model-derived autumn GPP. **b**, ORCHIDEE model-derived autumn NPP. **c**, ORCHIDEE model-derived autumn NEP. **d**, Sum of satellite-derived autumn normalized difference vegetation index (NDVI). The sensitivity is expressed as the linearly regressed slope of autumn carbon flux or of NDVI against autumn temperature for each pixel over the past two decades. A positive slope of NEP indicates that terrestrial carbon uptake is increasing with warmer temperatures, and vice versa. Areas with a low sensitivity or insignificant ($P > 0.05$) relationships between the variables are coloured in grey.

impact of autumn versus spring warming on ecosystem carbon exchange contributes significant uncertainty to future projections. If warming in autumn occurs at a faster rate than in spring, the ability of northern ecosystems to sequester carbon may diminish in the future. Acquiring a greater understanding of responses of terrestrial ecosystems to climate trends at the edges of the growing season, including potential acclimation processes, is clearly a priority, and should come from controlled ecosystem experiments and long-term eddy-covariance data sets.

METHODS SUMMARY

We analysed the effects of autumn temperature on the carbon balance of northern ecosystems at different scales, using three different methods.

First, we used smoothed flask CO_2 data from the NOAA/ESRL network[28] to characterize changes in the seasonal CO_2 zero-crossing dates[18,27] for ten stations over the Northern Hemisphere (Fig. 1). We correlated each zero-crossing date with the corresponding observed temperature[5] or precipitation[5] in spring (March to May) and autumn (September to November), and with the ORCHIDEE[16]-modelled soil moisture content. The trends in CO_2 zero-crossing dates and their correlation with climate factors were computed by using linear least-squares regression. The significance of statistical analyses in this study were assessed on the basis of two-tailed significance tests. To isolate further the contribution of fluxes and transport to the year-to-year atmospheric CO_2 signal, we performed factorial simulation experiments in which NEP from the ORCHIDEE[16] vegetation model forced by varying climate fields[5] provided surface boundary conditions for simulated CO_2 in the atmospheric transport model LMDZt[17] driven by interannual winds.

Second, we analysed the net CO_2 flux data measured by the eddy-covariance technique from 24 northern ecosystem sites (Supplementary Table 4). The end of the CUP is defined as the last day in a year when the NEP five-day running means exceed zero. Autumn is defined as the interval of ±30 days around the average CUP ending date at each site. We grouped the 108 year-site data into distinct 0.5 °C bins of autumn temperature anomaly. For each autumn temperature bin we calculated the median and mean anomaly of the ending date of the CUP.

Third, hints on the processes that control the integrated autumn NEP response to temperature, through the individual sensitivity of photosynthesis and respiration, were provided by integrating the ORCHIDEE vegetation model[16] forced by historic climate data[5] during the period 1980–2002.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 16 April; accepted 6 November 2007.

1. Goulden, M. L. *et al.* Sensitivity of boreal forest carbon balance to soil thaw. *Science* 279, 214–217 (1998).
2. Randerson, J. T., Field, C. B., Fung, I. Y. & Tans, P. P. Increases in early season ecosystem uptake explain recent changes in the seasonal cycle of atmospheric CO_2 at high northern latitudes. *Geophys. Res. Lett.* 26, 2765–2768 (1999).
3. Morgenstern, K. *et al.* Sensitivity and uncertainty of the carbon balance of a Pacific Northwest Douglas-fir forest during an El Niño La Niña cycle. *Agric. For. Meteorol.* 123, 201–219 (2004).
4. Bergeron, O. *et al.* Comparison of carbon dioxide fluxes over three boreal black spruce forests in Canada. *Glob. Change Biol.* 13, 89–107 (2007).
5. Mitchell, T. D. & Jones, P. D. An improved method of constructing a database of monthly climate observations and associated high-resolution grids. *Int. J. Climatol.* 25, 693–712 (2005).
6. Myneni, R. B., Keeling, C. D., Tucker, C. J., Asrar, G. & Nemani, R. R. Increased plant growth in the northern high latitudes from 1981 to 1991. *Nature* 386, 698–702 (1997).
7. Zhou, L. M. *et al.* Variations in northern vegetation activity inferred from satellite data of vegetation index during 1981 to 1999. *J. Geophys. Res.* 106, 20069–20083 (2001).
8. Churkina, G., Schimel, D., Braswell, B. H. & Xiao, X. M. Spatial analysis of growing season length control over net ecosystem exchange. *Glob. Change Biol.* 11, 1777–1787 (2005).
9. Cramer, W. *et al.* Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. *Glob. Change Biol.* 7, 357–373 (2001).
10. Sitch, S. *et al.* Evaluation of the terrestrial carbon cycle, future plant geography and climate–carbon cycle feedbacks using 5 Dynamic Global Vegetation Models (DGVMs). *Glob. Change Biol.* (in the press).
11. Black, T. A. *et al.* Increased carbon sequestration by a boreal deciduous forest in years with a warm spring. *Geophys. Res. Lett.* 27, 1271–1274 (2000).
12. Tanja, S. *et al.* Air temperature triggers the recovery of evergreen boreal forest photosynthesis in spring. *Glob. Change Biol.* 9, 1410–1426 (2003).
13. Menzel, A. *et al.* European phenological response to climate change matches the warming pattern. *Glob. Change Biol.* 12, 1969–1976 (2006).
14. Schwartz, M. D., Ahas, R. & Aasa, A. Onset of spring starting earlier across the Northern Hemisphere. *Glob. Change Biol.* 12, 343–351 (2006).
15. IPCC. *Climate Change 2007: The Physical Sciences Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, Cambridge, 2007).
16. Krinner, G. *et al.* A dynamic global vegetation model for studies of the coupled atmosphere–biosphere system. *Glob. Biogeochem. Cycles* 19, doi:10.1029/2003GB002199 (2005).
17. Hourdin, F. & Armengaud, A. The use of finite-volume methods for atmospheric advection of trace species. Part I: Test of various formulations in a general circulation model. *Mon. Weath. Rev.* 127, 822–837 (1999).
18. Keeling, C. D., Chin, J. F. S. & Whorf, T. P. Increased activity of northern vegetation inferred from atmospheric CO_2 measurements. *Nature* 382, 146–149 (1996).
19. Heimann, M. *et al.* Evaluation of terrestrial carbon cycle models through simulations of the seasonal cycle of atmospheric CO_2: First results of a model intercomparison study. *Glob. Biogeochem. Cycles* 12, 1–24 (1998).
20. GLOBALVIEW-CO2. *Cooperative Atmospheric Data Integration Project Carbon Dioxide*, (ftp.cmdl.noaa.gov/ccg/co2/GLOBALVIEW) (2004).
21. Angert, A. *et al.* Drier summers cancel out the CO_2 uptake enhancement induced by warmer springs. *Proc. Natl Acad. Sci. USA* 102, 10823–10827 (2005).
22. Linderholm, H. W. Growing season changes in the last century. *Agric. For. Meteorol.* 137, 1–14 (2006).
23. Ciais, P. *et al.* Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437, 529–533 (2005).
24. Piao, S. L., Friedlingstein, P., Ciais, P., Zhou, L. M. & Chen, A. P. Effect of climate and CO_2 changes on the greening of the Northern Hemisphere over the past two decades. *Geophys. Res. Lett.* 33, doi:10.1029/2006GL028205 (2006).
25. Myneni, R. B. & Song, X. *NDVI Version 3 based on Pathfinder*, (ftp://primavera.bu.edu/pub/datasets/AVHRR_DATASETS/PATHFINDER/VERSION3_DATA/) (2003).
26. Marti, O. *et al.* *The New IPSL Climate System Model: IPSL-CM4* (Institut Pierre Simon Laplace, Paris, 2006).
27. Thoning, K. W., Tans, P. P. & Komhyr, W. D. Atmospheric carbon-dioxide at Mauna Loa Observatory 2. Analysis of the NOAA GMCC data, 1974–1985. *J. Geophys. Res.* 94, 8549–8565 (1989).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank all the people and their respective funding agencies who worked to provide data for this study, and specifically B. Amiro, M. A. Arain, T. A. Black, C. Bourque, L. Flanagan, J. H. McCaughey and S. Wofsy for providing some of the flux data from the Canadian sites, and M.-A. Giasson and C. Coursolle for their help in compiling the data. We also thank A. Friend, P. Rayner and N. Viovy for helpful comments and discussions. This study was supported by European Community-funded projects ENSEMBLES and CARBOEUROPE IP, and by the National Natural Science Foundation of China as well as by Fluxnet-Canada, which was supported by CFCAS, NSERC, BIOCAP, MSC and NRCan. The computer time was provided by CEA. We thank the NOAA-ERSL global air sampling program for collecting and analysing the long-term CO_2 flask data, and K. Masarie at NOAA-ERSL for generating each year the GLOBALVIEW-CO_2 collaborative data product, which formed the basis of our atmospheric data analysis. The ongoing exchange of ideas, data and model results in the international research community on the carbon cycle is facilitated by the Global Carbon Project.

Author Contributions P.C., S.P., P.F. and P.P. designed the research. S.P., P.C. and P.F. performed ORCHIDEE modelling analysis. P.P. and S.P. performed transport analysis. S.P., S.L., M.R., H.M. and P.C. performed eddy-covariance data analysis. S.P., P.C. and J.F. performed satellite data analysis. All authors contributed to the interpretation and writing.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.L.P. (slpiao@lsce.ipsl.fr) or P.C. (philippe.ciais@lsce.ipsl.fr).

LETTERS

Vertical structure of recent Arctic warming

Rune G. Graversen[1], Thorsten Mauritsen[1], Michael Tjernström[1], Erland Källén[1] & Gunilla Svensson[1]

Near-surface warming in the Arctic has been almost twice as large as the global average over recent decades[1–5]—a phenomenon that is known as the 'Arctic amplification'. The underlying causes of this temperature amplification remain uncertain. The reduction in snow and ice cover that has occurred over recent decades[6,7] may have played a role[3,8]. Climate model experiments indicate that when global temperature rises, Arctic snow and ice cover retreats, causing excessive polar warming[9–11]. Reduction of the snow and ice cover causes albedo changes, and increased refreezing of sea ice during the cold season and decreases in sea-ice thickness both increase heat flux from the ocean to the atmosphere. Changes in oceanic and atmospheric circulation, as well as cloud cover, have also been proposed to cause Arctic temperature amplification[12–17]. Here we examine the vertical structure of temperature change in the Arctic during the late twentieth century using reanalysis data. We find evidence for temperature amplification well above the surface. Snow and ice feedbacks cannot be the main cause of the warming aloft during the greater part of the year, because these feedbacks are expected to primarily affect temperatures in the lowermost part of the atmosphere, resulting in a pattern of warming that we only observe in spring. A significant proportion of the observed temperature amplification must therefore be explained by mechanisms that induce warming above the lowermost part of the atmosphere. We regress the Arctic temperature field on the atmospheric energy transport into the Arctic and find that, in the summer half-year, a significant proportion of the vertical structure of warming can be explained by changes in this variable. We conclude that changes in atmospheric heat transport may be an important cause of the recent Arctic temperature amplification.

The recent warming of the Earth's surface is most probably due to an increase of atmospheric greenhouse-gas concentrations[8]. Although most greenhouse gases are fairly uniformly distributed around the globe, the temperature response to greenhouse-gas forcing is thought to be larger in polar than equatorial regions[10]. The response depends on various feedbacks within the climate system. In addition to snow and ice processes, the strength of the atmospheric stratification constitutes such a feedback. The troposphere is more stably stratified in the polar regions than closer to the Equator. An increase in downwelling long-wave radiation at the surface (for example, due to an altered atmospheric CO_2 level) causes warming, which at high latitudes is confined to the lower troposphere[18]. In the tropics, in contrast, the warming is distributed vertically by deep convection. It has also been proposed that the increase of polluting materials (such as black carbon) on Arctic ice and snow have caused albedo changes and added to the Arctic warming[19]. Common to all these processes is that they are expected to induce the largest warming in the lowermost part of the atmosphere.

The Arctic amplification can also be caused by other processes. Idealized experiments with models that have no surface-albedo feedback also reveal a polar-temperature-amplification response to a doubling of CO_2 concentration[12]. It is found that the excessive Arctic warming is due to an increase of the atmospheric northward transport of heat and moisture. These results are supported by observational studies, which suggest that changes of the heat transport have added to the recent Arctic surface warming[20].

The linkage between Arctic warming and changes of atmospheric circulation has been investigated by studying various Northern Hemisphere circulation indices, such as that associated with the Arctic Oscillation[21]. Generally, different phases of these indices are associated with linear temperature responses characterized by east–west heat redistribution between the mid-latitude ocean and land, whereas the high latitudes are less affected. However, in the winter season, high phases of the circulation indices are associated with a warmer Arctic. This warming is particularly pronounced over the northern rims of the continents[13–16]. From the 1970s through to the mid-1990s, the indices were in their high phases, while since then, they have relaxed towards neutral values. The Arctic warming, on the other hand, has shown a persistently positive trend over the past 30 years. It is therefore difficult to associate changes in these indices,

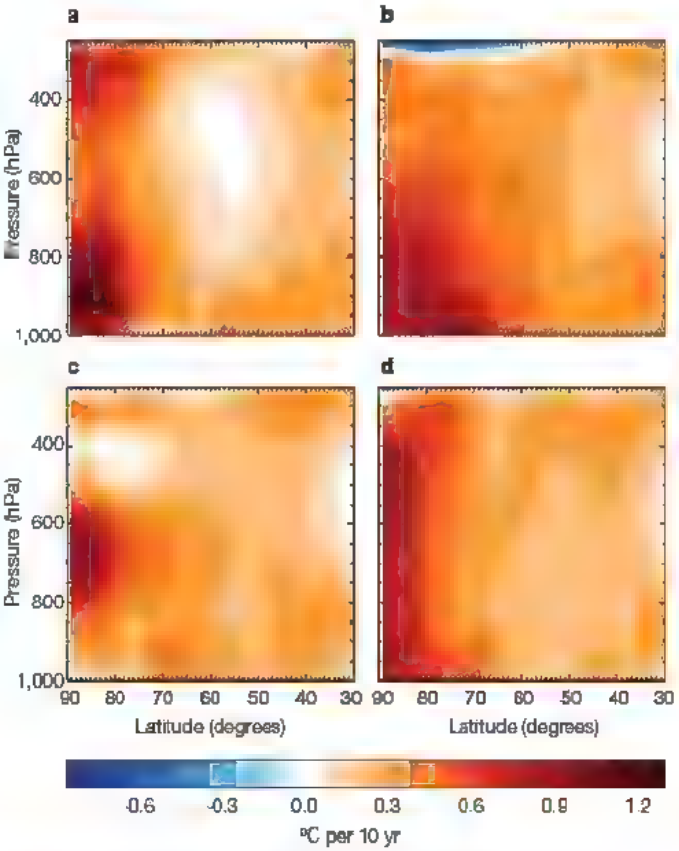


Figure 1 | Averaged temperature trends around latitude circles for 1979–2001 plotted versus latitude and height for the four seasons. Trends are shown for winter (**a**, December–February), spring (**b**, March–May), summer (**c**, June–August) and autumn (**d**, September–November). The linear trends are estimated from monthly mean data using a least-squares fit

[1]Department of Meteorology, Stockholm University, S-106 91 Stockholm, Sweden.

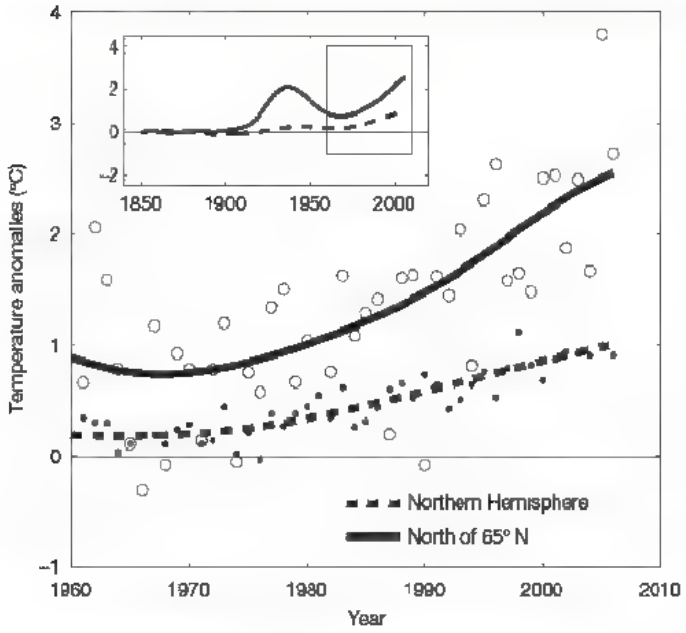


Figure 2 | Dark-month (November–February) anomalies of mean temperature relative to the 1850–1900 average as function of year. Data were obtained from land-station observations. In the main figure, the symbols represent means from individual years, whereas the lines show the temporal evolution when variability over timescales smaller than 20 years has been removed using a wavelet filter. Solid line and open circles are based on observations north of 65° N, while the dashed line and dots are for the entire Northern Hemisphere. Inset shows the smoothed temperature time series for the full instrumental period. The data were provided by the Climate Research Unit (CRU) as a 5° × 5° gridded data set[24]

such as the Arctic Oscillation index, with the recent Arctic warming trend.

The vertical structure of the Arctic warming during the 1980s and 1990s, based on the ERA-40 reanalysis (see Methods), exhibits trends throughout large parts of the troposphere that are comparable in magnitude to those at the surface (Fig. 1). In fact, the Arctic warming in the reanalysis data shows clear maxima well above the surface in winter and in summer, and the trends are almost equal at heights below the 400 hPa level in the atmosphere during autumn. This vertical structure is not consistent with the hypothesis that retreating snow and ice cover is the main cause of the amplification. Retreating snow and ice are associated with energy input at the surface, which—along with the stable stratification conditions often prevailing in the Arctic—means that this process would be expected to induce the largest temperature response in the lowermost part of the troposphere. But we only observe this vertical structure of warming in spring. It is worth noting that this is when the trends above the boundary layer are of comparable magnitude to those at the surface. We note that the lack of amplification near the surface in summer is consistent with expectations because surface air temperatures over the Arctic Ocean are constrained to be close to the freezing point owing to the melting of sea ice, but that the amplification aloft cannot be explained by surface feedbacks.

It is also notable that during the same period observations solely from Arctic land stations reveal an amplification of the temperature trend during the dark months, November–February (Fig. 2). This amplification cannot be explained by snow-cover changes, as the albedo effect is practically absent during this dark period. Moreover, the heat flux from the ground is very small. This is contrary to conditions in the ice-free parts of the Arctic oceans during winter, where convection ensures that cold water at the surface is replaced by warmer water from below; this process maintains a large vertical temperature gradient between the ocean surface and the cold atmosphere. In addition, reduction of sea-ice cover during summer results in increased sea-ice formation during autumn and winter, such that latent heat is stored during the warm season and released into the atmosphere during the subsequent months.

So what are the mechanisms giving rise to the vertical structure of the Arctic warming? Changes in the advection of atmospheric energy into the Arctic region might imply Arctic warming with a maximum not necessarily located at the surface. Mid-tropospheric temperatures in the Arctic are sensitive to advection of energy across the Arctic boundary: this is evident from linear regressions of the Arctic 500 hPa temperature field on the atmospheric northward energy transport (ANET) across 60° N (Fig. 3). Positive (negative) anomalies of the ANET at 60° N are followed by positive (negative) temperature anomalies over the Arctic area, where the anomalies of

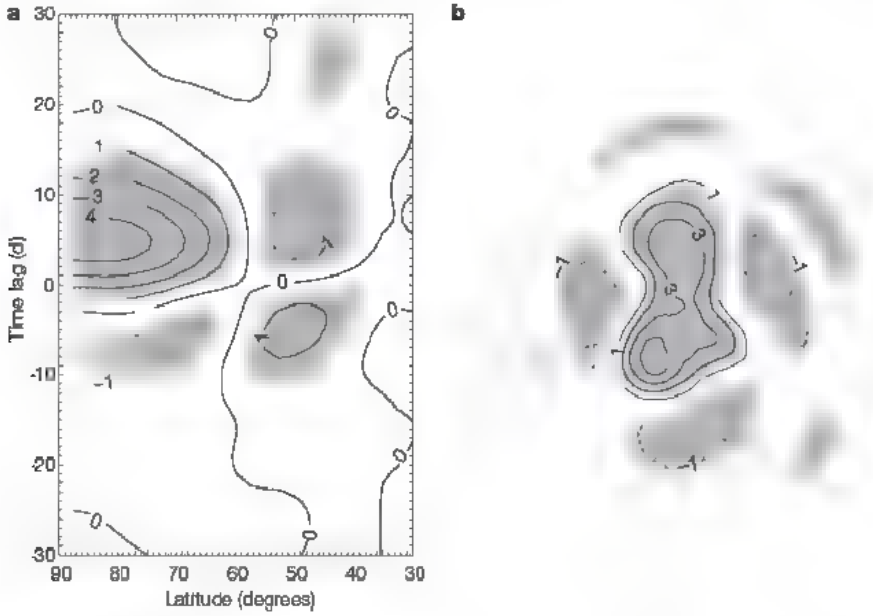


Figure 3 | Regressions of the 500 hPa temperature field on the atmospheric northward energy transport (ANET) across 60° N. a, Regressions averaged around latitude circles as a function of latitude and time lag; **b,** regressions for 5-day lag as a function of longitude and latitude. Solid and dotted contours indicate positive and negative regressions, respectively. In each point the regression has been scaled by the spatial standard deviation of all regressions. Light- and dark-grey shading shows areas where regressions differ significantly from zero at the 99% and 99.9% level, respectively. The regressions indicate temperature anomalies associated with an ANET anomaly at lag zero. For instance, a positive ANET anomaly is followed 5 days later by warming and cooling north and south of 60° N, respectively.

the temperatures lag those of the ANET by about 5 days (Fig. 3a). Positive regressions are found for positive time lag north of 60° N and for negative time lag south of 60° N, whereas the opposite distribution is found for negative regressions. In a statistical sense, this indicates that large energy transport follows conditions where a larger-than-usual north–south temperature gradient at 60° N has prevailed. This transport, in turn, is succeeded by warming of areas north of 60° N and cooling south of this latitude—a signature of energy convergence and divergence north and south of 60° N, respectively. The warming evaluated at five-day lag (Fig. 3b) is distributed over the major part of the Arctic area, whereas the cooling in the mid-latitudes is found over large parts of the continents. This linkage between the ANET across 60° N and the temperature field is found through the entire vertical extent of the troposphere (not shown), and is similar to that found from regressions of the surface temperatures on the ANET[20].

The ANET across 60° N has increased during recent decades, except in January and February. For the summer half-year, April through to October, the ANET can explain a substantial part of the Arctic temperature trends (Fig. 4). The part of the temperature trends that can be linked to the ANET (Fig. 4b) shows roughly the same vertical distribution as the total temperature trends (Fig. 4a), with a maximum at around 700 hPa. At 60° N, the ANET is mainly accomplished by atmospheric waves, such as Rossby waves and cyclone systems. Hence, the ANET at mid-latitudes can be viewed as an index of atmospheric circulation patterns. This index appears to be an efficient indicator of a linkage between circulation changes and Arctic temperature trends.

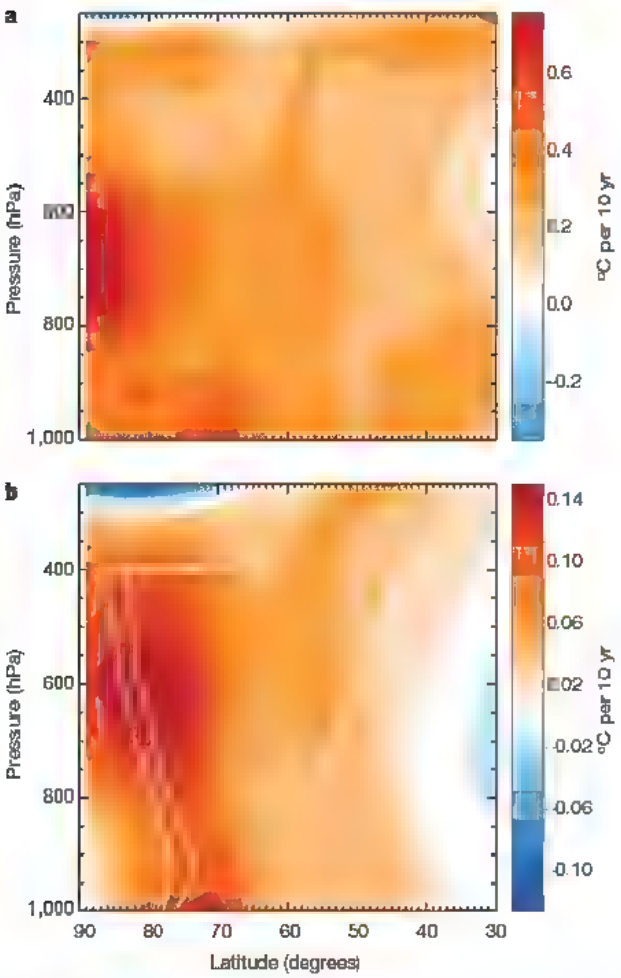


Figure 4 | Averaged temperature trends around latitude circles for 1979–2001 plotted versus latitude and height for April–October. **a**, Total trends; **b**, trends that are linked to the ANET across 60° N. The shadings indicate trends, and the white contours indicate areas where trends differ significantly from zero at the 99% and 99.9% level, respectively

Other processes that might be important contributors to the warming above the surface include changes in cloud cover and the atmospheric water vapour content. In the Arctic, except possibly for a short summer period, persistent low clouds are believed to induce surface warming[22]. Often the greenhouse effect of clouds dominates over the albedo effect, as the clouds cover an already highly reflecting surface. By absorbing radiation, clouds may furthermore warm the atmosphere at the height where they are present. As a result, it is possible that an increase in cloud cover at a given atmospheric height may cause warming there. Observations from satellites indeed suggest an increase of Arctic cloud fraction in summer during the 1980s and 1990s[17]. Warming of the Arctic middle troposphere might also partly be an effect of changes in the atmospheric radiative properties. These changes could be associated with the above-mentioned increase in advection of energy, which is basically a transport of warm and/or moist air into the Arctic. The advection in itself accounts for a considerable part of the maximum warming at 700 hPa, but additional warming at this height would occur if the advected air is more humid than the ambient air and hence absorbs long-wave radiation more efficiently; water vapour is an efficient greenhouse gas[8].

Our results do not imply that studies based on models forced by anticipated future CO_2 levels are misleading when they point to the importance of the snow and ice feedbacks. It is likely that a further substantial reduction of the summer ice-cover would strengthen these feedbacks and they could become the dominant mechanism underlying a future Arctic temperature amplification. Much of the present warming, however, appears to be linked to other processes, such as atmospheric energy transports.

METHODS SUMMARY

The ERA-40 reanalysis[23] data are used for Figs 1, 3 and 4. A discussion of the data quality and a comparison with two other reanalysis data sets are given in the Supplementary Discussion. The ANET at a given latitude is defined as the total energy flux across this particular latitude. Hence, this quantity constitutes one time series. Using daily data, the temperature field has been regressed on the ANET at 60° N for different time lags of the temperature field relative to the ANET (Fig. 3). When these regressions are multiplied by the ANET time series, projections of the temperature field on the ANET are obtained. On the basis of monthly mean data, linear trends of these projections have been estimated (Fig. 4) using a least squares fit. A Monte Carlo approach with a large number of artificial ANET time series has been used in order to estimate the significance of the results in Figs 3 and 4.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 28 March; accepted 29 November 2007.

1. Simon, C., Arris, L. & Heal, B. *Arctic Climate Impact Assessment* (Cambridge Univ. Press, New York, 2005)
2. Johannessen, O. M. *et al.* Arctic climate change: observed and modeled temperature and sea-ice variability. *Tellus A* 56, 328–341 (2004)
3. Polyakov, I. V. *et al.* Observationally based assessment of polar amplification of global warming. *Geophys. Res. Lett.* 29, doi:1029/2001GL011111 (2002)
4. Rigor, I. G., Colony, R. L. & Martin, S. Variations in surface air temperature observations in the Arctic, 1979–97. *J. Clim.* 13, 896–914 (2000).
5. Serreze, M. C. & Francis, J. A. The Arctic amplification debate. *Clim. Change* 76, 241–264 (2006)
6. Cavalieri, D. J., Gloersen, P., Parkinson, C. L., Comiso, J. C. & Zwally, H. J. Observed hemispheric asymmetry in global sea ice changes. *Science* 278, 1104–1106 (1997)
7. Stroeve, J. C. *et al.* Tracking the Arctic's shrinking ice cover: Another extreme September minimum in 2004. *Geophys. Res. Lett.* 32, doi:10.1029/2004GL021810 (2005)
8. Solomon, S., *et al.* (eds) *Climate Change 2007: The Physical Science Basis* (Cambridge Univ. Press, Cambridge, UK, 2007)
9. Holland, M. M. & Bitz, C. M. Polar amplification of climate in coupled models. *Clim. Dyn.* 21, 221–232 (2003).
10. Hansen, J. *et al.* Efficacy of climate forcing. *J. Geophys. Res.* 110, doi:10.1029/2005JD005776 (2005)
11. Chapman, W. L. & Walsh, J. E. Simulation of Arctic temperature and pressure by global coupled models. *J. Clim.* 20, 609–632 (2007)
12. Alexeev, V. A., Langen, P. L. & Bates, J. R. Polar amplification of surface warming on an aquaplanet in "ghost forcing" experiments without sea ice feedbacks. *Clim. Dyn.* 24, 655–666 (2005).

13. Thompson, D. W. J. & Wallace, J. M. Regional climate impacts of the Northern Hemisphere annular mode and associated climate trends. *Science* 293, 85–89 (2001).
14. Moritz, R. E., Bitz, C. M. & Steig, E. J. Dynamics of recent climate change in the Arctic. *Science* 297, 1497–1502 (2002).
15. Wu, Q. & Straus, D. M. AO, COWL, and observed climate trends. *J. Clim.* 17, 2139–2156 (2004).
16. Quadrelli, R. & Wallace, J. M. A simplified linear framework for interpreting patterns of Northern Hemisphere wintertime climate variability. *J. Clim.* 17, 3728–3744 (2004).
17. Wang, X. & Key, J. R. Arctic surface, cloud, and radiation properties on the AVHRR polar pathfinder dataset. Part II: Recent trends. *J. Clim.* 18, 2575–2593 (2005).
18. Manabe, S. & Weatherald, R. T. The effect of doubling the CO_2 concentrations on the climate of a general circulation model. *J. Atmos. Res.* 32, 3–15 (1975).
19. Hansen, J. *et al.* Dangerous human-made interference with climate: a GISS modelE study. *Atmos. Chem. Phys.* 7, 2287–2312 (2007).
20. Graversen, R. G. Do changes in the midlatitude circulation have any impact on the Arctic surface air temperature trend? *J. Clim.* 19, 5422–5438 (2006).
21. Thompson, D. W. J. & Wallace, J. M. The Arctic Oscillation signature in the wintertime geopotential height and temperature fields. *Geophys. Res. Lett.* 9, 1297–1300 (1998).
22. Intrieri, J. M. *et al.* An annual cycle of Arctic surface cloud forcing at SHEBA. *J. Geophys. Res.* 107, doi:10.1029/2000JC000439 (2002).
23. Uppala, S. M. *et al.* The ERA-40 re-analysis. *Q. J. R. Meteorol. Soc.* 131, 2961–3012 (2005).
24. Jones, P. D., New, M., Parker, D. E., Martin, S. & Rigor, I. G. Surface air temperature and its variations over the last 150 years. *Rev. Geophys.* 37, 173–199 (1999).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank P. Lundberg for comments on the manuscript. The ERA-40 data were obtained from the European Centre for Medium-Range Weather Forecasts (ECMWF) data server, whereas the Climate Research Unit (CRU) at the University of East Anglia provided the observational data used for Fig. 2.

Author Contributions The analysis was performed and the manuscript written by R.G.G., and to some extent T.M. The original idea to use ERA-40 data to study Arctic warming was due to R.G.G., M.T. and E.K. All authors contributed with ideas, discussions and text.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.G.G. (rune@misu.su.se).

LETTERS

Effects of acoustic waves on stick–slip in granular media and implications for earthquakes

Paul A. Johnson[1], Heather Savage[2,3], Matt Knuth[2,4], Joan Gomberg[5] & Chris Marone[2]

It remains unknown how the small strains induced by seismic waves can trigger earthquakes at large distances, in some cases thousands of kilometres from the triggering earthquake, with failure often occurring long after the waves have passed[1–6]. Earthquake nucleation is usually observed to take place at depths of 10–20 km, and so static overburden should be large enough to inhibit triggering by seismic-wave stress perturbations. To understand the physics of dynamic triggering better, as well as the influence of dynamic stressing on earthquake recurrence, we have conducted laboratory studies of stick–slip in granular media with and without applied acoustic vibration. Glass beads were used to simulate granular fault zone material, sheared under constant normal stress, and subject to transient or continuous perturbation by acoustic waves. Here we show that small-magnitude failure events, corresponding to triggered aftershocks, occur when applied sound-wave amplitudes exceed several microstrain. These events are frequently delayed or occur as part of a cascade of small events. Vibrations also cause large slip events to be disrupted in time relative to those without wave perturbation. The effects are observed for many large-event cycles after vibrations cease, indicating a strain memory in the granular material. Dynamic stressing of tectonic faults may play a similar role in determining the complexity of earthquake recurrence.

Laboratory studies of granular friction have emerged as a powerful tool for investigating tectonic fault zone processes and earthquake phenomena, including post-seismic slip, interseismic frictional restrengthening and earthquake nucleation[7,8]. Here we explore experimentally the effects of dynamic loading on stick–slip behaviour and discuss how our results may affect understanding of earthquake processes—in particular dynamic earthquake triggering and stick–slip recurrence. Dynamic earthquake triggering involves seismic waves from one earthquake promoting or inhibiting failure on the faults they disturb. Dynamic triggering has been clearly documented in a few cases far from an earthquake source, at distances much greater than the fault radius of the triggering source[1–4,6] (outside the traditional 'aftershock zone'), and increasing evidence suggests that it commonly occurs near the earthquake source[5,9].

Experiments on sheared layers of glass beads (like those shown in Fig. 1, and described in the Methods section) exhibit stick–slip that varies with shear displacement rate, confining stress, relative humidity, granular media thickness, and particle characteristics[10–12]; however, for fixed experimental conditions stick–slip characteristics are remarkably constant (Fig. 2). Stick–slip events are characterized by sudden, periodic shear stress drops that range from 10–30% of the maximum frictional strength. Leading up to steady-state strength, which takes several tens of seconds and shear strains of ~0.4–0.5, we observe a material dilation and nonlinear shear-stress increase accompanied by intermittent failure. During steady-state frictional behaviour, major stick–slip events recur very regularly but include rare, small events (for example, at 1,375 s in Fig. 2b). Each major stick–slip event is followed by elastic and then inelastic stress build-up and layer dilation. The dilation is manifested by increasing layer thickness (Fig. 2b). Layers dilate to a point of instability at which catastrophic dynamic failure and compaction occur (Fig. 2b)

The top curve of Fig. 3a shows results from an experiment identical to that of Fig. 2 except that we applied acoustic waves during shearing, commencing a few seconds before expected stick–slip failure, and continuing until the major failure event. The lower curve of Fig. 3a shows the rectified peak strain amplitude measured by the accelerometer attached to the sample (Fig. 1), along with three

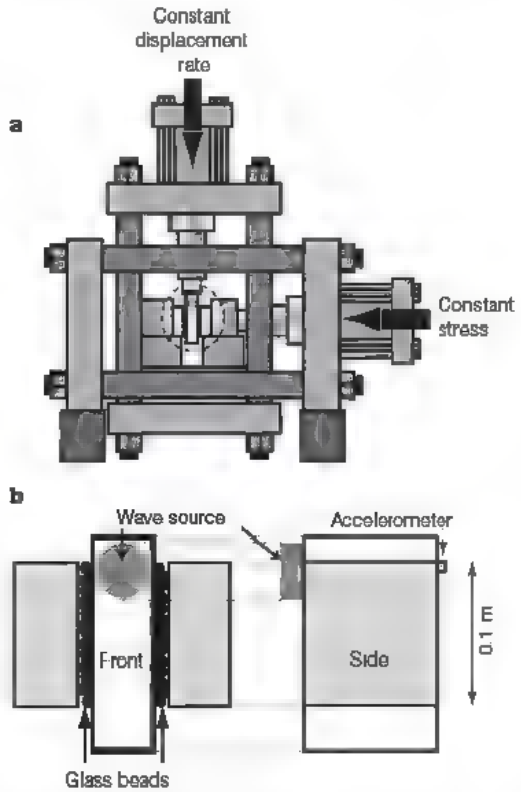


Figure 1 | Experimental apparatus. a, Apparatus, showing horizontal piston applying constant normal stress, and vertical piston applying a constant (vertical) displacement rate, which drives shear. The dashed circle shows the sample assembly. **b**, Sample assembly showing three-block arrangement of the double-direct shear configuration (front and side views). We note the location of the acoustic wave source and accelerometer in relation to the glass bead layers and normal stress (horizontal).

[1]Geophysics Group EES-11, Los Alamos National Laboratory of the University of California, MS D443, Los Alamos, New Mexico 87545, USA. [2]Department of Geosciences, Pennsylvania State University, University Park, Pennsylvania 16802, USA. [3]Department of Earth and Planetary Science, University of California, Santa Cruz, California 95064, USA. [4]Department of Geology and Geophysics, University of Wisconsin, Madison, Wisconsin 53706, USA. [5]US Geological Survey, University of Washington, Department of Earth and Space Sciences, Box 351310, Seattle, Washington 98195-1310, USA

intervals for which dynamic waves were applied and signals from acoustic emission from both small and large stick–slip failures.

Vibration perturbs the recurrence period of inelastic stress increase before the failure of major events and induces small-amplitude stick–slip events. In many cases one or more small stick–slip events occur during vibration, as well as cascades of delayed, small-amplitude stick–slip events (Fig. 3a, grey shading). In all cases, application of acoustic waves—even for brief intervals—has a lasting effect, such that successive major stick–slip events exhibit a strain memory of applied vibration manifest by delayed failure, disruption of recurrence interval and extended aseismic creep, despite the violent mechanical re-set that occurs during major stick–slip events (Fig. 3). We find that post-vibration, the regular recurrence does not recover.

We also apply acoustic pulses, rather than the longer-duration waves described above. Pulses are more analogous to a single seismic wave in Earth, whereas vibration may be more analogous to the near-source region where quasi-continuous-wave energy may exist for significant periods of time in the form of aftershocks. Our data show that continuous and pulse modes of dynamic triggering yield similar behaviour. See Supplementary Fig. 1, where we show a typical sequence of stick–slip events in the presence of acoustic pulses.

When we apply vibration or pulsed sound at stresses below ~95% of the failure strength there is little or no effect on stick–slip. This implies that the system must be in a critical state to be susceptible to dynamic triggering, which is consistent with seismic data on earthquake triggering[13] and recent modelling[14]. Qualitatively, wave strain amplitudes must exceed approximately 10^{-6} for the above effects to be observed (Supplementary Fig. 2), consistent with dynamic triggering observations for real earthquakes[15]. When the system is driven with vibration amplitudes corresponding to strains $< 10^{-6}$ there is no obvious effect on stick–slip; however, we emphasize that this should be further quantified in future experiments

Analysis of the primary stick–slip recurrence intervals for otherwise identical experiments with and without vibration shows that failure becomes progressively more erratic and, on average, lengthens with time in experiments with wave excitation (Fig. 4a). Repeated experiments with both vibration and pulse-mode verify that this effect is real. We find that the scatter relative to the mean increases with accumulated time in experiments with vibration. Also, although the primary stick–slip recurrence interval increases significantly due to acoustic waves, the stress-drop magnitude and variation increase only slightly (Fig. 4b).

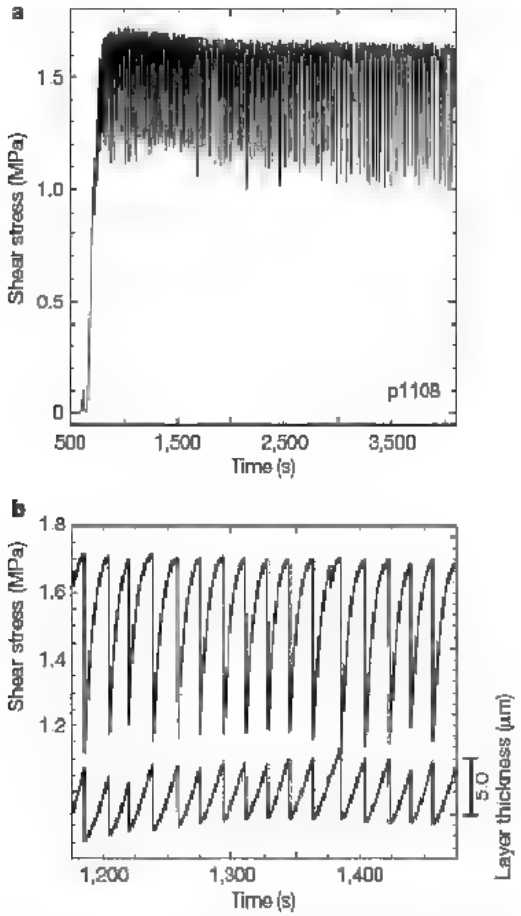


Figure 2 | Stick–slip behaviour under constant shearing rate, without vibration. **a**, Shear stress versus experiment time for a typical run. Note that maximum stick–slip stress drops are ~30% of the shear strength. Over the total duration of the experiment, there is a small but progressive compaction of about 1% of the glass bead layer thickness (not shown). **b**, Detail of the stick–slip cycles (top) and change in layer thickness (bottom). The layer thickness has had the overall trend removed. We note consistent failure strength, recurrence interval, and creep before stick–slip. p1108 refers to experiment number.

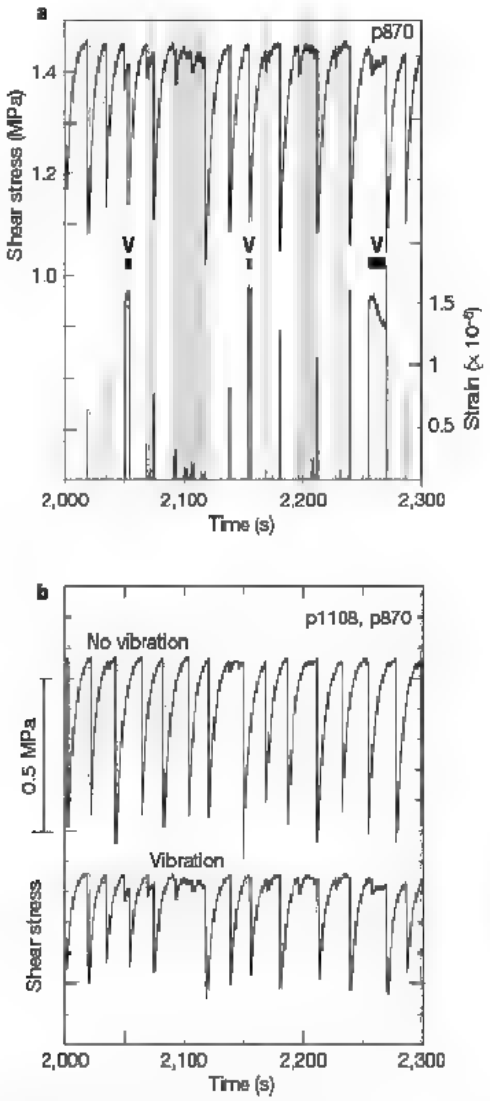


Figure 3 | Stick–slip with and without vibration. **a**, Stick–slip behaviour under constant shearing rate, with vibration. Shear stress versus experiment time (upper curve), and measured, rectified strain amplitudes of the detected acoustic waves (lower curve). The letter 'V' denotes times and thick black horizontal bars indicate the durations of vibration. Vibration has a marked influence on the stick–slip behaviour. For instance, the applied vibration at ~2,050 s produces an immediate, small-magnitude stick–slip. The two successive major stick–slips that follow exhibit longer recurrence times as well as multiple small stick–slip events in between—these are triggered events. Regions of triggered events are shaded light grey. Similarly, irregular cycles occur following the applied vibration at 2,155 s. Vibration applied at ~2,255 s produces an immediate small-magnitude stick–slip event and an increased major-event recurrence interval. **b**, Comparison of non-vibration versus vibration, emphasizing increased recurrence and irregular behaviour, including triggering, due to acoustic waves. p870 and p1108 refer to experiment numbers.

We have described three primary experimental observations: (1) acoustic waves disrupt recurrence intervals and, to a lesser degree, stress drops of large magnitude events; (2) acoustic waves trigger immediate and delayed small-magnitude events, some aseismic; and (3) strain memory of acoustic perturbation is maintained through successive large-magnitude stick–slips. We assess the implications of these results for dynamic earthquake triggering by considering that the primary slick–slip events represent tectonic earthquakes and that the vibration-induced events represent triggered earthquakes.

The overall trend of increasing stress drop (and maximum frictional strength) with recurrence interval is consistent with a large body of previous laboratory and field observations[8] showing that maximum frictional strength increases linearly with the log of recurrence interval between slip events. Vibration diminishes the rate at which stress drop increases with inter-event time, notably creating greater irregularity in stick–slip recurrence interval. The commonly used class of rate-state frictional models explicitly predicts that the rate of strengthening is proportional to the product of the normal stress and the frictional constitutive parameters[8]. Because we hold the normal stress constant, this implies that vibration alters frictional properties, despite the fact that perturbation amplitudes ($\sim 10^4$ Pa) are less than 1% of the normal load. We suggest that the irregularity in recurrence that we observe in our experiments mimics that observed for tectonic faults in the Earth's crust, and reflects a complex process of disrupting the internal fault zone structure.

We find that vibration has measurable effects only when the system is in a critical state, approaching failure (for example, see Fig. 3a).

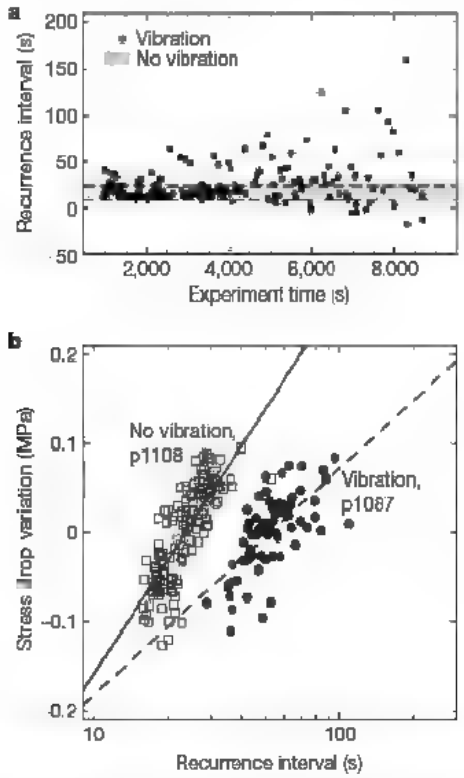


Figure 4 | Stick-slip recurrence time and stress drop comparing vibration and non-vibration experiments. **a**, Recurrence versus experiment time for runs with vibration (solid circles) and without. The shaded region and dashed lines show the mean recurrence interval of ±1 standard deviation. Data trend removed. Compared to the non-vibration experiments, both the scatter and average recurrence interval increases progressively in experiments with vibration. **b**, Stress-drop variation versus recurrence for experiments conducted with and without vibration. We cannot compare stress-drop amplitudes directly owing to minor differences from one experiment to the next; however, when we compare the variation of stress drop to the experimental mean, we see a clear trend of longer recurrence interval for a given change in stress drop.

Application of acoustic waves also has a measurable effect only for experiments conducted at relatively small normal stresses, approximately 4–5 MPa. We have explored higher horizontal loads (up to ~18 MPa) for which we did not see a vibrational effect; perhaps the vibration amplitude was not sufficiently large to produce an effect. Nevertheless, the laboratory experiments do imply that dynamic earthquake triggering at seismic strain amplitudes is most efficient at low effective stress (normal load minus pore pressure) for faults in a critical state. Some field-based studies also point to a connection between earthquake triggering and low effective stress and/or faults near failure[6,16,17], although this is a point of some debate[18].

One mystery regarding dynamic earthquake triggering is that it can take place minutes, hours or days after the seismic perturbation. Our experiments show delayed failures following acoustic perturbations, frequently manifesting as cascades of small events. We do not yet understand the physics responsible for this observation; however, we speculate that triggered events, as well as the recurrence and stress-drop disruptions are manifestations of frictional contact mechanics coupled with granular processes. Previous work shows that stick–slip initiates as failure of a contact junction between beads in highly stressed chains of particles[11,12]. Granular memory effects are presumably the result of similar processes.

We posit that acoustic waves disrupt granular force chains, leading to material softening and simultaneous weakening (granular flow), similar to what is described in a recently proposed phenomenological model[19]. The manifestation of the acoustic disruption may take place immediately or later in time (strain 'memory'). The vibration-induced memory itself may be maintained as frictional instability at a number of grain contacts that persist through one or more stick–slip cycles, and is reminiscent of dynamically induced strain memory, known as 'slow dynamics', observed in nonlinear dynamical experiments on glass bead packs[19]. The memory is also suggestive of statically induced rate-dependent effects observed in sheared granular materials, such as 'ageing'[7,20]. We attempted to erase vibration-induced memory by ceasing shear loading to allow the material to heal, as well as by changing normal stress to repack the grains, but neither approach succeeded.

Our previous work shows that permanent damage to the grains themselves is negligible[12] and therefore cannot be the origin of the behaviours observed. Moreover, acoustical studies in three-dimensional glass bead packs under similar wave strain amplitudes, and under (smaller) static stresses of 0.02–0.1 MPa, show no evidence for grain rearrangement; however, the material exhibits very small, irreversible compaction as well as nonlinear-induced modulus softening and slow dynamics[21]. Hertz–Mindlin contact mechanics describe these observations[21]. The compaction we measure in our experiments without vibration is small and does not lead to instability. The addition of vibration shows additional compaction but it is extremely small. Taken together, the observations suggest that minute compaction plays a part in what we observe, but there is no clear evidence suggesting that it is the cause. Our data do not rule out the possibility that instability is abetted, or initiated, by localized compaction (for example, within a shear band in the layer[22]), which would be invisible to our measurements. Local compaction within a granular material would reduce normal stress at contact junctions, which could lead to stick–slip instability. For the moment, the origin of what we observe when stick–slip is combined with vibration remains unknown.

The origin of dynamic earthquake triggering by transient seismic waves is a complex problem. Our results show that granular-friction processes are consistent with two as-yet-unexplained observations in earthquake seismology: (1) small-amplitude waves can trigger both immediate failure and delayed failure relative to the strain transient, and (2) earthquake recurrence patterns are complex. Understanding the role of vibration-induced disruption of earthquake recurrence could have significant implications for seismic hazard assessment and reliable forecasting of earthquakes.

METHODS SUMMARY

In our experimental study of acoustic waves interacting with a laboratory-scale fault system, we employ a double-direct shear configuration to shear 4-mm layers of glass beads at constant normal stress (1–18 MPa), using shearing rates of 1–100 $\mu m\,s^{-1}$ (Fig. 1). Class IV bead dimensions are 105–149 μm in diameter. Layers are subject to either continuous vibration or wave pulses of 10–20 cycles at 1–20 kHz, with strain amplitudes ranging from $<5 \times 10^{-7}$ to 8×10^{-6}, or alternatively, to no wave excitation. An acoustic source and accelerometer are mounted directly on the central shearing block (Fig. 1b). We measure stresses, displacements and wave-induced strains continuously throughout shearing.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 23 July; accepted 31 October 2007.

1. Hill, D. P. *et al.* Seismicity remotely triggered by the magnitude 7.3 Landers, California, earthquake. *Science* **260**, 1617–1623 (1993).
2. Gomberg, J., Bodin, P., Larson, K. & Dragert, H. Earthquake nucleation by transient deformations caused by the $M = 7.9$ Denali, Alaska earthquake. *Nature* **427**, 621–624 (2004).
3. Brodsky, E., Karakostas, V. & Kanamori, H. A. New observation of dynamically triggered regional seismicity: Earthquakes in Greece following the August, 1999, Ismit, Turkey earthquake. *Geophys. Res. Lett.* **27**, 2741–2744 (2000).
4. Hough, S. E. Triggered earthquakes and the 1811–1812 New Madrid, Central United States earthquake sequence. *Bull. Seismol. Soc. Am.* **91**, 1574–1581 (2001).
5. Gomberg, J., Bodin, P. & Reasenberg, P. A. Observing earthquakes triggered in the near field by dynamic deformations. *Bull. Seismol. Soc. Am.* **93**, 118–138 (2003).
6. Brodsky, E. & Prejean, S. G. New constraints on mechanisms of remotely triggered seismicity at Long Valley Caldera. *J. Geophys. Res.* **110**, 4302–4316 (2005).
7. Marone, C. Laboratory-derived friction laws and their application to seismic faulting. *Ann. Rev. Earth Planet. Sci.* **26**, 643–696 (1998).
8. Scholz, C. H. *The Mechanics of Earthquakes and Faulting* 2nd edn (Cambridge Univ. Press, 2002).
9. Felzer, K. & Brodsky, E. Evidence for dynamic aftershock triggering from earthquake densities. *Nature* **441**, 735–738 (2006).
10. Nasuno, S., Kudrolli, A., Bak, A. & Gollub, J. P. Time-resolved studies of stick–slip friction in sheared granular layers. *Phys. Rev. E* **58**, 2161–2171 (1998).
11. Mair, K., Frye, K. M. & Marone, C. Influence of grain characteristics on the friction of granular shear zones. *J. Geophys. Res.* **107**, ECV4-1–9 (2002).
12. Anthony, J. L. & Marone, C. Influence of particle characteristics on granular friction. *J. Geophys. Res.* **110**, B08409, doi:10.1029/2004JB003399 (2005).
13. Freed, A. M. Earthquake triggering by static, dynamic, and postseismic stress transfer. *Annu. Rev. Earth Planet. Sci.* **33**, 335–367 (2005).
14. Ziv, A. What controls the spatial distribution of remote aftershocks? *Bull. Seismol. Soc. Am.* **96**, 2231–2241 (2007).
15. Gomberg, J. & Johnson, P. A. Dynamic triggering of earthquakes. *Nature* **473**, 830 (2005).
16. Zoback, M. D. & Beroza, G. Evidence for near-frictionless faulting in the 1989 (M 6.9) Loma Prieta, California, earthquake and its aftershocks. *Geology* **21**, 181–185 (1993).
17. Beeler, N. M. & Lockner, D. A. Why earthquakes correlate weakly with the solid Earth tides: effects of periodic stress on the rate and probability of earthquake occurrence. *J. Geophys. Res.* **108**, 2391–2408 (2003).
18. Scholz, C. H. Evidence for a strong San Andreas fault. *Geology* **28**, 163–166 (2000).
19. Johnson, P. A. & Jia, X. Nonlinear dynamics, granular media and dynamic earthquake triggering. *Nature* **473**, 871–874 (2005).
20. Hartley, R. R. & Behringer, R. P. Logarithmic rate dependence of force networks in sheared granular materials. *Nature* **421**, 928–931 (2003).
21. Brunet, T., Jia, X. & Johnson, P. Transitional, elastic-nonlinear behaviour in dense granular media. *Geophys. Res. Lett.* (submitted).
22. Morgan, J. K. & Boettcher, M. S. Numerical simulations of granular shear zones using the distinct element method: 1. Shear zone kinematics and the micromechanics of localization. *J. Geophys. Res.* **104**, 2703–2719 (1999).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Funding was provided by Institutional Support (LDRD) at Los Alamos and the DOE Office of Basic Energy Science (P.A.J.), by the National Science Foundation (C.M., H.S., M.K.), and by the United States Geological Survey (J.G.). We thank E. Brodsky, B. Behringer, N. Beeler and X. Jia for comments and reviews.

Author Contributions P.A.J., M.K., H.S. and C.M. designed the study. M.K., P.A.J. and C.M. designed and carried out the data collection procedure. P.A.J. and H.S. did most of the data analyses. P.A.J. and C.M. did most of the writing. P.A.J., H.S., M.K. and C.M. did the laboratory work and J.G. and C.M. did much of the writing interpretation. All authors contributed to the interpretation and writing.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to P.A.J. (paj@lanl.gov).

LETTERS

Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice

Daniel Huber[1,2], Leopoldo Petreanu[1,2], Nima Ghitani[1], Sachin Ranade[2], Tomáš Hromádka[2], Zach Mainen[2] & Karel Svoboda[1,2]

Electrical microstimulation can establish causal links between the activity of groups of neurons and perceptual and cognitive functions[1–6]. However, the number and identities of neurons microstimulated, as well as the number of action potentials evoked, are difficult to ascertain[7,8]. To address these issues we introduced the light-gated algal channel channelrhodopsin-2 (ChR2)[9] specifically into a small fraction of layer 2/3 neurons of the mouse primary somatosensory cortex. ChR2 photostimulation *in vivo* reliably generated stimulus-locked action potentials[10–13] at frequencies up to 50 Hz. Here we show that naive mice readily learned to detect brief trains of action potentials (five light pulses, 1 ms, 20 Hz). After training, mice could detect a photostimulus firing a single action potential in approximately 300 neurons. Even fewer neurons (approximately 60) were required for longer stimuli (five action potentials, 250 ms). Our results show that perceptual decisions and learning can be driven by extremely brief epochs of cortical activity in a sparse subset of supragranular cortical pyramidal neurons.

We used *in utero* electroporation[14] to introduce ChR2 fused to a green fluorescent protein (GFP) (ChR2–GFP[15]) together with a red fluorescent cytosolic marker[15] (RFP) into neocortical pyramidal neurons (Fig. 1a, Methods). In the adult brain, ChR2–GFP expression was restricted to pyramidal cells in layers 2/3 (more than 99.4%), mainly in the barrel cortex (Figs 1a and 2a). *In vivo* two-photon imaging and retrospective immunohistology revealed that ChR2–GFP was localized to the neuronal plasma membrane. ChR2–GFP was expressed in about half (48.9 ± 5.3%, $n = 10$, five mice; see Methods) of red fluorescent layer 2/3 neurons (Supplementary Movie 1). ChR2–GFP invaded the soma, dendrites and axons (Fig. 1b, c). ChR2–GFP expression was stable for at least 8 months and did not seem to perturb neuronal morphology (Fig. 1a–c, Methods).

We next characterized the responses of ChR2–GFP-expressing neurons to photostimulation in anaesthetized mice. To sample from the entire population of ChR2–GFP-expressing neurons, unbiased by ChR2–GFP expression level, we recorded from red fluorescent neurons using two-photon targeted loose-patch recordings[16] (Fig. 1c, d). Photostimuli consisted of light pulses, produced by a blue miniature light-emitting diode (LED; 470 nm), centred on the recording window (Fig. 1d). At maximum light intensities ($I_{max} = 11.6$ mW mm^{-2} at the surface of the brain, centred on the diode; 1–10 ms duration) about half (51%) of the patched red neurons ($n = 39/77$, eight mice) responded reliably to single photostimuli with at most one action potential. Increasing the photostimulus duration beyond 10 ms did not reveal additional responsive neurons. The other half of the patched neurons did not fire spikes time-locked to the photostimuli, and presumably corresponded to ChR2–GFP-negative neurons. These measurements indicate that most ChR2–GFP-positive neurons can be driven to spiking using our photostimulation system; furthermore, excitation of layer 2/3 neurons through indirect synaptic pathways was weak.

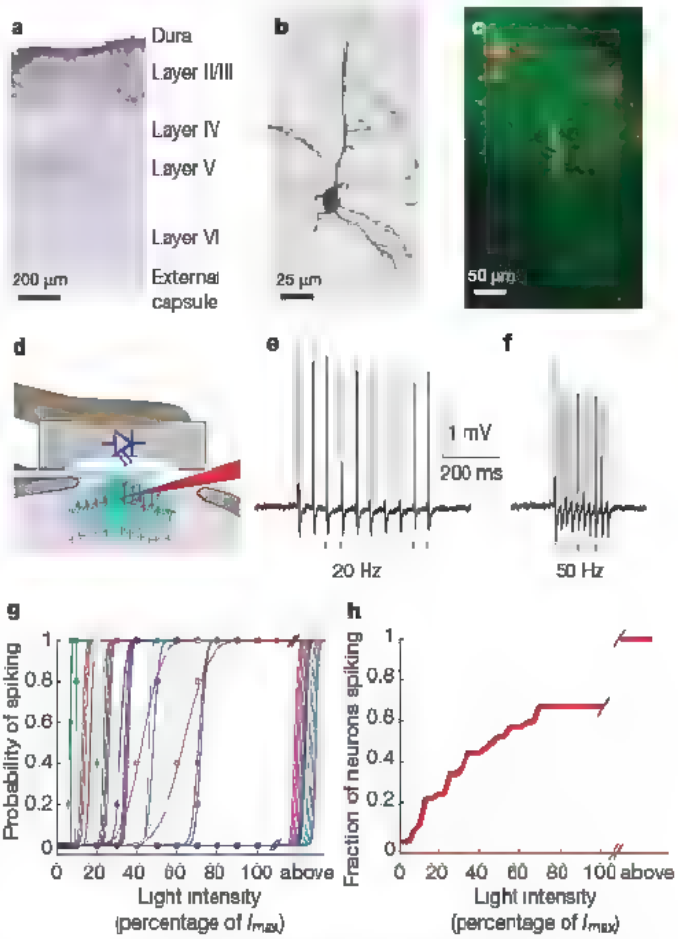


Figure 1 | ChR2-assisted photostimulation of layer 2/3 barrel cortex neurons *in vivo*. **a**, Coronal section through the electroporated mouse somatosensory cortex after immunohistochemical staining for ChR2–GFP **b**, Individual layer 2/3 neuron, side view **c**, Maximum value projection (top view) of an image stack *in vivo* (see Supplementary Movie 1) showing layer 2/3 neurons expressing ChR2–GFP and cytosolic RFP **d**, Schematic of the recording geometry **e**, Action potentials recorded from one ChR2–GFP-positive neuron. Blue bars indicate photostimuli (1 ms duration, 11.6 mW mm^{-2}, 20 Hz) **f**, Same as **e**, 50 Hz. **g**, Probability of spiking as a function of light intensity (1 ms duration five repetitions per condition, 15 s between stimuli) ($I_{max} = 11.6$ mW mm^{-2}) Each line corresponds to a different neuron, each colour to a different animal. Neurons that could only be driven with photostimuli longer than 1 ms were pooled at the far right (above). **h**, Cumulative fraction of recorded neurons firing at various threshold intensity levels (computed from the data in **g**)

[1]Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, Virginia 20147, USA. [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

When stimulated with 1 ms light pulses, ChR2–GFP-expressing neurons were able to follow frequencies up to 20 Hz (Fig. 1e) and in some cases up to 50 Hz (Fig. 1f). These frequencies are comparable to, or higher than, typical spike rates in the barrel cortex[17]. Action potentials followed the photostimuli with short delays (range 3–11 ms) and little jitter (Supplementary Fig. 1).

We next determined the relation between photostimulus intensity and the probability of spiking of ChR2–GFP-expressing neurons. During cell-attached recordings we stimulated with 1 ms light pulses while varying the photostimulus. With decreasing light intensity, neurons switched abruptly from firing action potentials with high probability to firing no action potentials. The photostimulus intensity required to trigger action potentials varied substantially across the population of ChR2–GFP-expressing neurons (Fig. 1g). Control experiments in brain slices revealed that the brightness of ChR2–GFP measured in individual cells was inversely correlated with firing threshold (Supplementary Fig. 2); in contrast, the firing threshold was independent of the depth of the recorded neuron *in vivo* (Supplementary Fig. 3). The variability in firing threshold in terms of photostimulus intensity therefore primarily reflects heterogeneity in the expression level of ChR2–GFP in individual neurons. These results confirm that ChR2 can transduce photostimuli into precisely timed spike trains *in vivo*[18]. Furthermore, the fraction of activated neurons can be tuned by modulating the excitation light intensity (Fig. 1h).

Can awake mice learn to report photostimulation of layer 2/3 pyramidal neurons in the barrel cortex? To address this question we delivered light pulses to ChR2–GFP-expressing neurons in freely moving animals (Fig. 2a). We first implanted a window above the barrel cortex[19], which provided optical access for photostimulation and screening the density of electroporated neurons. We next mounted the miniature LED centred on the imaging window (Fig. 2a; Methods). During the behavioural sessions the mice were temporarily connected to an LED controller (Methods). Mice were trained in a detection task to associate photostimulation of ChR2–GFP-expressing neurons (five light pulses, 20 Hz, 1 ms duration) with water reward on one of two choice ports (Fig. 2b, left port). After four to seven training sessions (200–800 trials per session) all animals expressing ChR2–GFP ($n = 9$) reliably reported photostimulation; in the presence (absence) of a photostimulus, mice chose the left (right) port (Fig. 3a, range 72–93% correct, defined as hits + correct rejections, divided by total number of trials; Supplementary Movie 2). Control mice without electroporated neurons ($n = 6$) performed at chance levels (50.1%, $P > 0.70$, t-test), even after 25 training sessions (Fig. 3a and Supplementary Fig. 4). These experiments demonstrate that photostimulation of layer 2/3 neurons can drive robust behaviour.

How many action potentials triggered by photostimulation are necessary for perception? To address this issue we further trained five mice to respond to one, two and five photostimuli at 20 Hz (example in Fig. 2c). Although performance decreased with fewer pulses, all ChR2–GFP-expressing mice were able to detect single action potentials in the activated cells, even at modest photostimulus intensities (Fig. 3b, red lines).

To determine the relation between performance and the number of neurons directly activated by light, we measured behaviour as a function of light intensity (Fig. 3b). As expected, behavioural performance decreased with decreasing photostimulus intensity, although the psychometric curves varied from animal to animal. For example, at the lowest intensities probed (10% of I_{max}) some animals continued to discriminate, whereas others performed at chance levels.

We counted the number of ChR2–GFP-positive somata and measured their positions (Fig. 3c; Methods). Between 594 and 1430 ChR2–GFP-positive neurons were found in a 2 mm diameter window (Fig. 3b, c; Methods). The number of ChR2–GFP-positive

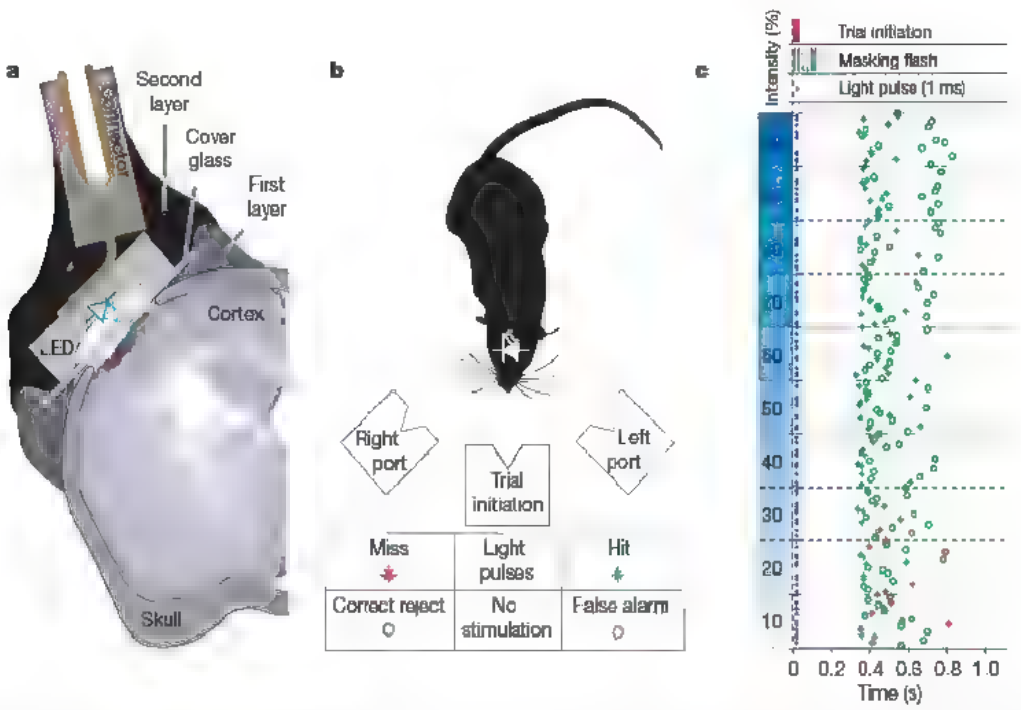


Figure 2 | Photostimulation in freely moving mice performing a detection task. **a**, Schematic of the photostimulation setup (see Methods) **b**, Schematic of the behavioural apparatus and reward contingencies. The mouse initiates a trial by sticking its snout into the centra. port. Photostimuli are applied during a stimulation period (300 ms) accompanied by a series of bright blue light flashes delivered to the behavioural arena (30 Hz, 300 ms) to mask possible scattered light from the portable light source. The mouse then decides to enter either the left or the right port for a water reward. If a photostimulus was present, the choice of the left port was rewarded with a drop of water (hit, green star) whereas the choice of the right port lead to a short timeout (4 s, miss, red star). If the stimulus was absent, only the choice of the right port was rewarded with reward (correct reject, green circle) whereas the left port lead to a timeout (4 s, false alarm, red circle). **c**, Data from one session (200 trials) with a single stimulus (1 ms) with decreasing light intensities. Each horizontal line delineates 20 trials at fixed light intensity. Blue dots indicate the presence or absence of a photostimulus. Stimulated and non-stimulated trials were presented pseudo-randomly with a probability of 0.5.

neurons under the photostimulation window correlated with the performance of individual animals.

For each animal we then estimated the number of active neurons as a function of normalized intensity (I_o = intensity/I_{max}) as:

$$N_a(I_o) = \sum_{k \in \text{cells}} f(I_o i(r_k)) \quad (1)$$

Here r_k is the horizontal position of the kth ChR2–GFP-positive cell and f is the fraction of ChR2-positive cells activated at intensity $I_o i$ (Fig. 1h). $i(r)$ is the spatial distribution of the normalized light intensity in the tissue (horizontal full-width at half maximum = 2.17 mm, 250 μm below the pia) (Supplementary Methods; Supplementary Fig. 5). For trains of five action potentials, an average of 61 neurons (range 6–197) was sufficient to drive reliable performance (more than 65% of correct choices), whereas 297 (range 135–381) active neurons were required with single action potentials (Fig. 3d). The total number of action potentials required for a given level of performance was independent of the stimulus pattern (Supplementary Fig. 6).

Two factors make us believe that our estimates of the number of active neurons should be interpreted as an upper bound. First, the measured spatial distribution of light in the tissue is likely broader than the actual distribution of light (see Supplementary Methods). Second, we did not consider possible deterioration of the optical path (thickening of the dura, bone growth, and so on) over the long timescales required for the behavioural experiments compared with the more favourable conditions of the calibration measurements. Therefore the actual number of activated neurons in the behavioural experiments might have been lower than the numbers cited above.

Not surprisingly, triggering more action potentials yields better detection accuracy (Fig. 3d). However, performance reached asymptotic levels at remarkably low numbers of directly activated neurons; the range between minimal detection and saturating performance was only a few hundred neurons.

Activated ChR2–GFP-positive neurons were distributed over most of the barrel cortex, with a smattering in adjacent sensory areas. The activated cortical region contains at least 40,000 layer 2/3 neurons (approximately 2,000 per barrel column, unpublished data) implying that synchronous action potentials in less than 1% of layer 2/3 neurons can be robustly perceived. These data imply that mechanisms exist to read out extremely sparse codes from primary sensory areas[6,20,21]. Because of convergence in the L2/3 → L5 pathway, it is possible that even fewer activated L5 cells could be detected by behaving mice. We also note that the detection threshold could vary considerably based on the state of the animal[6,22].

We have shown that ChR2-based optical microstimulation can be used to dissect the impact of precisely timed action potentials in a few genetically defined neurons on mammalian behaviour. Our data show that the favourable characteristics of ChR2 reported previously *in vitro*[10–12,15,23–25], *in vivo*[18] and in invertebrate systems[24,26]—including the ability to generate precisely timed action potentials—are

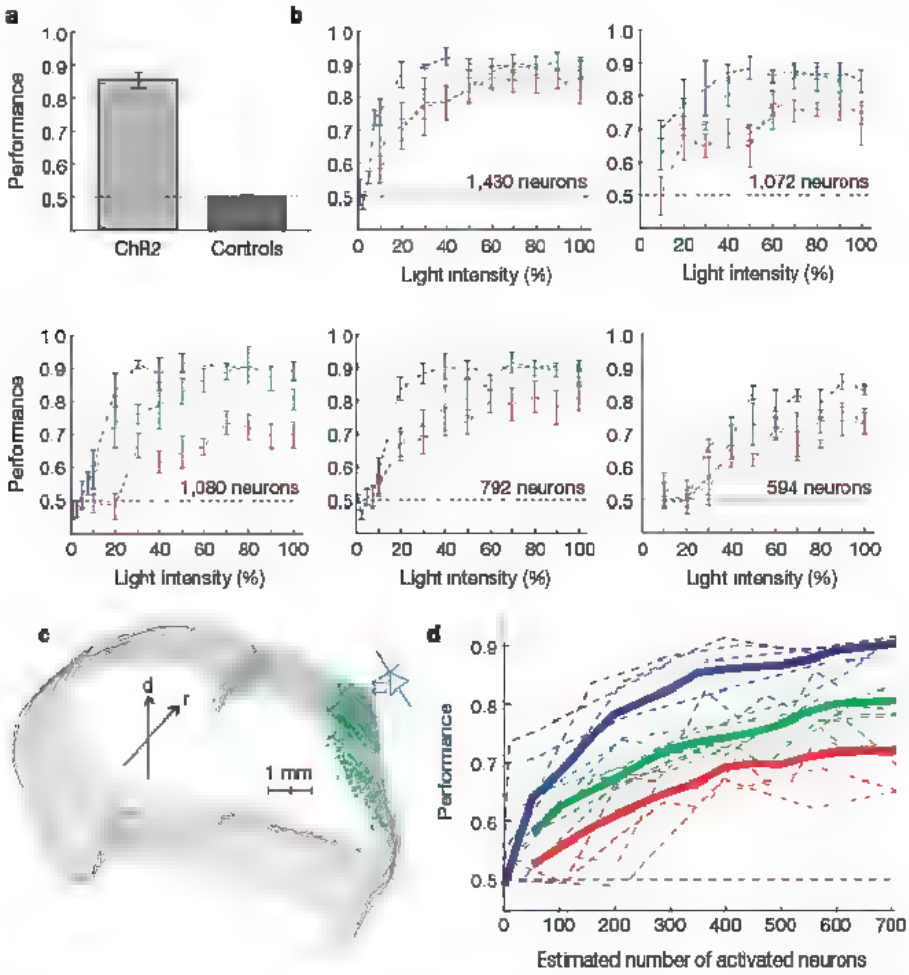


Figure 3 | Behavioural detection of photostimulation. a, Comparison of the performance ((hits + correct rejections)/total trials) in mice expressing ChR2–GFP ($n = 9$) and control mice ($n = 6$) after training with five photostimuli ($P < 0.001$, t-test). **b**, Performance as a function of light intensity (as percentage of I_{max} = 11.6 mW mm^{-2}) for five light pulses (1 ms, 20 Hz, blue lines), two light pulses (1 ms, 20 Hz, green lines) and a single light pulse (1 ms, red lines). Dotted lines: mean across five sessions (200–1000 trials per session). Error bars: binomial standard error. The number of ChR2–GFP-positive neurons located under the window area is indicated for each mouse. **c**, Location of ChR2-expressing neurons in serial reconstruction of the sectioned brain (corona. sections). The blue cone illustrates the light source over the window. Arrows indicate rostral (r) and dorsal (d) orientation. **d**, Performance as a function of the number of activated neurons. Thick lines, mean performance across all five animals for one (red), two (green) and five (blue) light pulses. Dotted lines indicate mean values of individual animals from Fig. 3b

maintained in awake conditions and can be used effectively to drive learning and behaviour.

Photostimulation of genetically defined neurons[27] has key advantages compared with electrical microstimulation. Under typical experimental conditions, electrical microstimulation excites axons non-discriminately, probably including diverse local and long-range axons[7,8]. Therefore, the cell type and cell location that drive behaviour in classical microstimulation experiments are poorly defined. Photostimulation of genetically defined neural populations naturally overcomes these problems. Our estimates of the number of directly activated cortical neurons necessary to drive perception is lower than previous estimates based on electrical microstimulation[28,29]. Our stimuli might be functionally more potent because a pure population of excitatory neurons is activated, whereas electrical microstimulation drives a mixture of diverse excitatory and inhibitory neurons. The robust associative learning induced by ChR2-assisted photostimulation opens the door to study the circuit basis of perception and cognition *in vivo*.

METHODS SUMMARY

***In utero* electroporation.** DNA solution (ChR2–GFP and either mCherry or DsRedexpress ('RFP'); 4:1 molar ratio; final concentration 2 μg μl^{-1}) was injected into the right lateral ventricle of embryonic mice (E16). Layer 2/3 progenitor cells were transfected by *in utero* electroporation[14,15].

Photostimulation and behaviour. An imaging window was implanted on the electroporated mice[19] at postnatal day 40–50. A miniature blue high-power LED (470 nm peak wavelength, NFSB036BT, Nichia, Japan) was mounted on the imaging window with black dental acrylic. The timing and intensity of the LED was computer-controlled with a custom-built, low-noise current-source circuit (see Methods). Mice were trained on a detection task to report photostimulation (see Methods). The training protocol consisted of several phases; transitions from one phase to the next were triggered by performance at 65% correct or above. Mice had restricted access to drinking water to maintain 80–85% of their pre-training weight. For calibrations using targeted cell-attached recordings[16] mice were placed under a custom-made two-photon laser-scanning microscope controlled by ScanImage software[19,30]. For the photostimulation the objective was removed and a miniature blue high-power LED (as described above) was placed on the centre of the recording window (see Methods).

Histology. After completion of behavioural experiments, the brain from each animal was cut into coronal or tangential sections (40–60 μm thick) on a cryostat (Leica, CM 3050S). The localization of ChR2–GFP-positive cell bodies was measured using Neurolucida software (MBF Bioscience).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 19 August; accepted 2 November 2007.
Published online 19 December 2007.

1. Penfield, W. & Boldery, P. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain* 60, 389–443 (1937).
2. Salzman, C. D., Britten, K. H. & Newsome, W. T. Cortical microstimulation influences perceptual judgements of motion direction. *Nature* 346, 174–177 (1990).
3. Romo, R., Hernandez, A., Zainos, A. & Salinas, E. Somatosensory discrimination based on cortical microstimulation. *Nature* 392, 387–390 (1998).
4. Libet, B. in *Handbook of Sensory Physiology* (ed. Iggo, A.) 743–790 (Springer, Berlin, 1973).
5. Leal-Campanario, R., Delgado-Garcia, J. M. & Gruart, A. Microstimulation of the somatosensory cortex can substitute for vibrissa stimulation during Pavlovian conditioning. *Proc. Natl Acad. Sci. USA* 103, 10052–10057 (2006).
6. Butovas, S. & Schwarz, C. Detection psychophysics of intracortical microstimulation in rat primary somatosensory cortex. *Eur. J. Neurosci.* 25, 2161–2169 (2007).
7. Tehovnik, E. J. Electrical stimulation of neural tissue to evoke behavioral responses. *J. Neurosci. Methods* 65, 1–17 (1996).
8. Ranck, J. B. Jr. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* 98, 417–440 (1975).
9. Nagel, G. *et al.* Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl Acad. Sci. USA* 100, 13940–13945 (2003).
10. Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G. & Deisseroth, K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neurosci.* 8, 1263–1268 (2005).
11. Li, X. *et al.* Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin. *Proc. Natl Acad. Sci. USA* 102, 17816–17821 (2005).
12. Ishizuka, T., Kakuda, M., Araki, R. & Yawo, H. Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels. *Neurosci. Res.* 54, 85–94 (2006).
13. Bi, A. *et al.* Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* 50, 23–33 (2006).
14. Hatanaka, Y., Hisanaga, S., Heizmann, C. W. & Murakami, F. Distinct migratory behavior of early- and late-born neurons derived from the cortical ventricular zone. *J. Comp. Neurol.* 479, 1–14 (2004).
15. Petreanu, L., Huber, D., Sobczyk, A. & Svoboda, K. Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nature Neurosci.* 10, 663–668 (2007).
16. Margrie, T. W. *et al.* Targeted whole-cell recordings in the mammalian brain *in vivo*. *Neuron* 39, 911–918 (2003).
17. Fee, M. S., Mitra, P. P. & Kleinfeld, D. Central versus peripheral determinants of patterned spike activity in rat vibrissa cortex during whisking. *J. Neurophysiol.* 78, 1144–1149 (1997).
18. Arenkiel, B. R. *et al. In vivo* light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron* 54, 205–218 (2007).
19. Gray, N. W., Weimer, R. M., Bureau, I. & Svoboda, K. Rapid redistribution of synaptic PSD-95 in the neocortex *in vivo*. *PLoS Biol.* 4, e370 (2006).
20. DeWeese, M. R., Wehr, M. & Zador, A. M. Binary spiking in auditory cortex. *J. Neurosci.* 23, 7940–7949 (2003).
21. Petersen, R. S., Panzeri, S. & Diamond, M. E. Population coding in somatosensory cortex. *Curr. Opin. Neurobiol.* 12, 441–447 (2002).
22. Ferezou, I., Bolea, S. & Petersen, C. C. Visualizing the cortical representation of whisker touch: voltage-sensitive dye imaging in freely moving mice. *Neuron* 50, 617–629 (2006).
23. Zhang, Y. P. & Oertner, T. G. Optical induction of synaptic plasticity using a light-sensitive channel. *Nature Methods* 4, 139–141 (2006).
24. Nagel, G. *et al.* Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr. Biol.* 15, 2279–2284 (2005).
25. Wang, H. *et al.* High-speed mapping of synaptic connectivity using photostimulation in channelrhodopsin-2 transgenic mice. *Proc. Natl Acad. Sci. USA* 104, 8143–8148 (2007).
26. Schroll, C. *et al.* Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr. Biol.* 16, 1741–1747 (2006).
27. Lima, S. Q. & Miesenbock, G. Remote control of behavior through genetically targeted photostimulation of neurons. *Cell* 121, 141–152 (2005).
28. Salzman, C. D., Murasugi, C. M., Britten, K. H. & Newsome, W. T. Microstimulation in visual area MT: effects on direction discrimination performance. *J. Neurosci.* 12, 2331–2355 (1992).
29. Tehovnik, E. J., Tolias, A. S., Sultan, F., Slocum, W. M. & Logothetis, N. K. Direct and indirect activation of cortical neurons by electrical microstimulation. *J. Neurophysiol.* 96, 512–521 (2006).
30. Pologruto, T. A., Sabatini, B. L. & Svoboda, K. ScanImage: flexible software for operating laser-scanning microscopes. *Biomed. Eng. Online* 2, 13 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank B. Burbach, D. Flickinger, H. Kessels, D. O'Connor, T. Sato, R. Weimer and A. Zador for help with experiments, and D. O'Connor for comments on the manuscript. This work was supported by the Swiss National Science Foundation (to D.H.), the National Institutes of Health and the Howard Hughes Medical Institute.

Author Contributions D.H. and K.S. designed the experiments. D.H. performed the behavioral and *in vivo* physiological experiments. L.P., D.H. and K.S. performed the brain slice measurements. N.G. performed histology. S.R., T.H., Z.M. and K.S. provided advice and equipment. D.H. and K.S. wrote the paper. All authors discussed the results and commented on the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to K.S. (svobodak@janelia.hhmi.org).

LETTERS

Behavioural report of single neuron stimulation in somatosensory cortex

Arthur R. Houweling[1,2] & Michael Brecht[1,2]

Understanding how neural activity in sensory cortices relates to perception is a central theme of neuroscience. Action potentials of sensory cortical neurons can be strongly correlated to properties of sensory stimuli[1] and reflect the subjective judgements of an individual about stimuli[2]. Microstimulation experiments have established a direct link from sensory activity to behaviour[3,4], suggesting that small neuronal populations can influence sensory decisions[5]. However, microstimulation does not allow identification and quantification of the stimulated cellular elements[6]. The sensory impact of individual cortical neurons therefore remains unknown. Here we show that stimulation of single neurons in somatosensory cortex affects behavioural responses in a detection task. We trained rats to respond to microstimulation of barrel cortex at low current intensities. We then initiated short trains of action potentials in single neurons by juxtacellular stimulation. Animals responded significantly more often in single-cell stimulation trials than in catch trials without stimulation. Stimulation effects varied greatly between cells, and on average in 5% of trials a response was induced. Whereas stimulation of putative excitatory neurons led to weak biases towards responding, stimulation of putative inhibitory neurons led to more variable and stronger sensory effects. Reaction times for single-cell stimulation were long and variable. Our results demonstrate that single neuron activity can cause a change in the animal's detection behaviour, suggesting a much sparser cortical code for sensations than previously anticipated.

Based on its volume[7] and density of neurons[8], rat somatosensory cortex contains an estimated two million neurons. The detection of single-cell stimulation might therefore be a difficult task, and we adopted a behavioural paradigm designed for observing single-cell effects (Fig. 1a). We first trained animals to report short (200 ms) trains of microstimulation pulses. Stimulation of somatosensory cortex evokes tactile sensations in humans[9], and animal studies have demonstrated an interchangeability of tactile stimuli and cortical microstimulation[10]. We mainly targeted deep cortical layers, where microstimulation detection thresholds are lowest in rat barrel cortex[11]. Tongue lick responses were rewarded with a drop of water and counted as a hit if a lick occurred within 100–1200 ms from stimulus onset (Fig. 1b). Animals typically learned this microstimulation report task in a single session and detection thresholds decreased to 2–5 μA within days (Supplementary Fig. 1). These values are comparable to the lowest cortical microstimulation detection thresholds reported in humans[12] and animals[11,13]. To be able to detect potentially weak effects of single-cell stimulation, we encouraged guessing (a non-conservative response criterion) by introducing only mild negative reinforcement (a 1.5 s time-out) for false-positive responses (licks without preceding stimulation).

Once animals responded consistently to low microstimulation currents, we approached a cortical neuron closely with a glass pipette and evoked short (200 ms) trains of action potentials by juxtacellular stimulation, a technique developed to label individual neurons[14]. Juxtacellular stimulation currents (3–43 nA, mean 12.6 nA) strongly modulated action potential firing in barrel cortex neurons (Fig. 1c, d). On average, we evoked 14.2 ± 6.5 (s.d.) action potentials during current injection, a 25-fold increase over the average spontaneous firing rate. The close apposition of neuron and pipette in the juxtacellular configuration in behaving animals typically resulted in short experimental sessions per cell. Microstimulation in the vicinity of the neuron (at an average distance of about 75 μm) was adjusted such

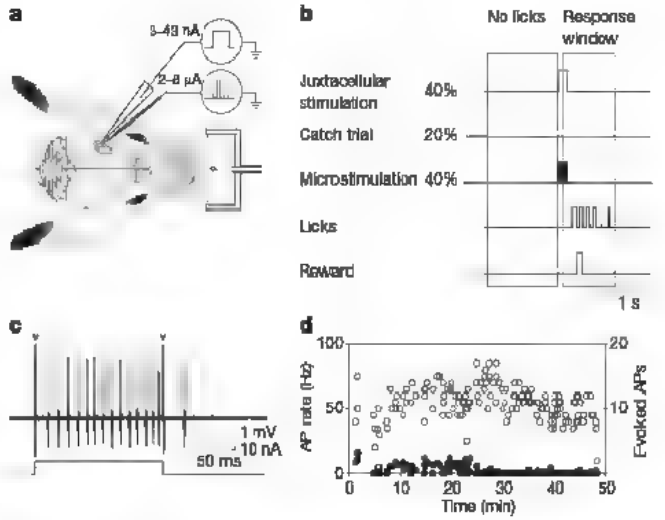


Figure 1 | Behavioural setup and single-cell stimulation. a, Stimulation experiments were performed in the barrel cortex of awake rats. Animals responded to stimulation by interrupting a light beam (dashed line) with multiple tongue licks. The time of the first lick was taken as the reaction time and reward was delivered for correct responses (right). Top, single-cell stimulation pipette with stimulation current wave form (upper) and tungsten microelectrode with stimulation pulse train (lower). **b,** Three types of stimulus were presented at random intervals (Poisson process, mean 3 s): microstimulation (2–8 μA) (40% probability), juxtacellular single-cell stimulation (40%) and no (or subthreshold) current injection 'catch' trials (20%). Licks within the interstimulus interval led to an additional 1.5 s delay to presentation of the next stimulus (left box) and were rewarded after a stimulus (right box) for all three trial types. **c,** Single-cell stimulation trial by juxtacellular current injection. Triangles indicate stimulation onset and offset artefacts. **d,** Evoked action potentials (open circles) in a series of stimulation trials. Spontaneous action potentials (solid circles) were quantified for 1 s before each stimulation. The left *y* axis label applies to both spontaneous and evoked action potentials; the right *y* axis label applies to evoked action potentials.

[1]Bernstein Center for Computational Neuroscience and Humboldt University Berlin, Philippstrasse 13 House 6, 10115 Berlin, Germany. [2]Department of Neuroscience, Erasmus Medical Center, PO Box 2040, 3000 CA, Rotterdam, The Netherlands

that current intensities were close to the animal's detection threshold, resulting in an average hit (detection) rate of 75%. The action potential firing of most cells was affected during and after microstimulation (Supplementary Fig. 2).

Microstimulation and single-cell stimulation trials were randomly interleaved with 'catch' trials (with no or subthreshold current injection) (Fig. 1b). In paradigms with random stimulus presentation times, catch trials can be used to estimate chance performance and to guard against inadvertent cues[15]. To assess single-cell effects when the animal was attentive, we confined our analysis to those single-cell stimulation and catch trials flanked by correct microstimulation responses.

Figure 2 shows an experiment on a regular spiking layer 5b pyramidal neuron with a slender apical dendrite (Fig. 2a). Juxtacellular stimulation evoked on average 9.1 action potentials during the current injection (Fig. 2b top). Lick responses (red squares) occurred mainly after single-cell stimulation (Fig. 2b top) and microstimulation (Fig. 2b bottom), but only once after no stimulation catch trials (Fig. 2b middle). Quantification of responses (Fig. 2c) suggests that the animal reported single pyramidal cell activity. Even though this neuron was one of the cells with the strongest behavioural effects, this effect was not significant on the single neuron level ($P = 0.099$, Fisher's exact test). This is not unexpected given the limited number of trials (see Methods).

A population analysis revealed, however, that single-cell stimulation biased animals towards responding. Figure 3a shows, for 51 neurons, that animals responded significantly more often in single-cell stimulation trials (mean hit rate 22.0%) than in no-current-injection catch trials (mean false-positive rate 17.9%; $P = 0.022$). To test if single-cell detection was dependent on the firing of the stimulated neuron rather than on inadvertent cues associated with the current injection, we stimulated a further set of 19 neurons. We applied single-cell stimulation as usual, but instead of the no-current catch trials we presented subthreshold (10% of the single-cell stimulation current) catch trials. Subthreshold current injections activated neurons only weakly or not at all. Animals also responded significantly more often in single-cell stimulation trials than in subthreshold catch trials (Fig. 3b; mean hit rate 27.4%; mean false-positive rate 20.6%; $P = 0.019$). Stimulation effects were distributed evenly across animals (Supplementary Fig. 3). Having verified that single-cell stimulation led to significant biases in two independent sets of neurons (Fig. 3a, b), we wanted to confirm that this effect did not result from the inadvertent stimulation of neighbouring neurons or other nonspecific effects. Thus, we injected current (25 nA, twice the average current applied with juxtacellular stimulation) through the pipette into extracellular space (instead of applying it to a neuron). These control experiments showed that animals did not report current injection into extracellular space (Fig. 3c; $n = 90$; mean hit rate 18.7%; mean false-positive rate 19.0%; $P = 0.598$). To test if single-cell stimulation effects (Fig. 3a, b) were different from those of extracellular current injection (Fig. 3c), we compared effect size (hit rate − catch trial response rate) across those two data sets and observed a significant difference ($P = 0.008$). Finally, we tested if single-cell stimulation effects were specific to the attended (and trained) cortical area. As before, microstimulation was applied to barrel cortex, but we now stimulated single neurons in visual cortex. Animals did not report single-cell stimulation in visual cortex (Fig. 3d, $n = 21$; mean hit rate 21.2%; mean false-positive rate 20.1%; $P = 0.319$), suggesting that stimulation effects are specific to the attended cortical area.

Further observations show that the animals' responses were caused by the stimulation of single and not multiple neurons. (1) Juxtacellular stimulation currents were approximately three orders of magnitude lower (3–43 nA) than those required for evoking motor or sensory responses with microstimulation (2–200 μA). (2) Although we occasionally observed the inadvertent stimulation of a second neuron by the appearance of a second large action potential waveform in our recordings, such inadvertent stimulation was rare (accounting for only about 1% of evoked action potentials across experiments; Supplementary Fig. 4). All results presented here were also significant when single-cell stimulation trials with secondary action potentials were excluded. (3) Firing rates of more distant cells (with action potentials less than 0.5 mV) were not modulated (Supplementary Fig. 5). (4) Juxtacellular labelling typically fills single neurons[14].

Because microstimulation in barrel cortex can evoke whisker movements, we combined stimulation experiments with whisker tracking to assess if rats sense single-cell stimulation indirectly by detecting movements. Our data argue against such an indirect mechanism: near detection threshold microstimulation did not evoke movements even though it was reported (Supplementary Fig. 6a) and single-cell stimulation did not evoke whisker movements (Supplementary Fig. 6b).

The bias towards responding evoked by single-cell stimulation was weak on average (approximate 5% effect size: single-cell stimulation hit rate − catch trial response rate). As illustrated in Supplementary Fig. 7, the strength of the effect depended greatly on the animal's overall response rate. When animals were conservative (low response

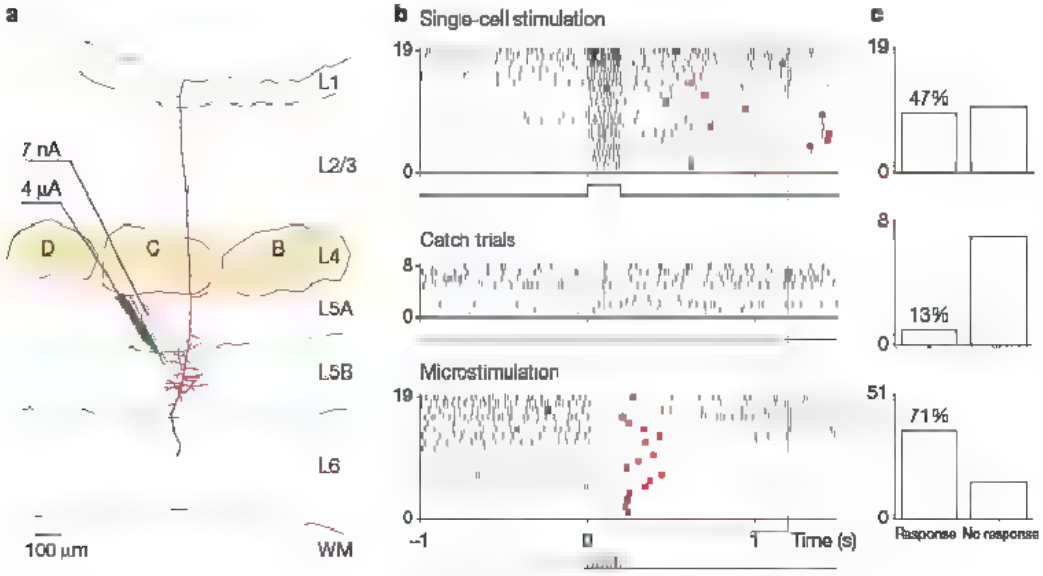


Figure 2 | Behavioural responses to stimulation of a single layer 5b pyramidal neuron. a, Reconstruction of the stimulated neuron with dendritic tree (red) and axon (blue, incompletely filled). Superimposed is a micrograph of a stimulation pipette and a tungsten microstimulation electrode aligned along the electrode track. Barrel rows (brown) are labelled with letters. L, layer; WM, white matter. **b**, Action potential (ticks) raster plots and first lick responses (red squares) during juxtacellular single-cell stimulation trials (top), no-current-injection catch trials (middle) and 19 randomly selected microstimulation trials (bottom). The neuron was inhibited during and after microstimulation (stimulation current, 4 μA). **c**, Quantification of responses to single-cell stimulation, catch trials and microstimulation.

rate) the effect was small (and not significant); at high response rates, however, the effect size was about 9% (and significant). Most interestingly, the variance of stimulation-evoked sensory effects was greater for putative interneurons than putative excitatory cells ($P = 0.0023$, see Methods), which we distinguished based on spike width and firing pattern (Supplementary Fig. 8). Whereas stimulation of putative excitatory neurons led to weak but significant biases towards responding, stimulation of putative inhibitory neurons led to stronger and more variable sensory effects (Fig. 3e). In particular, in 3 out of 11 putative interneurons the effect was stronger than in any of the 59 putative excitatory cells or 90 control experiments. In two of these three putative interneurons most hits were observed for trials in which the evoked firing rates were not higher than the population average, suggesting that interneuron action potentials are in some cases more readily detected than action potentials of excitatory cells.

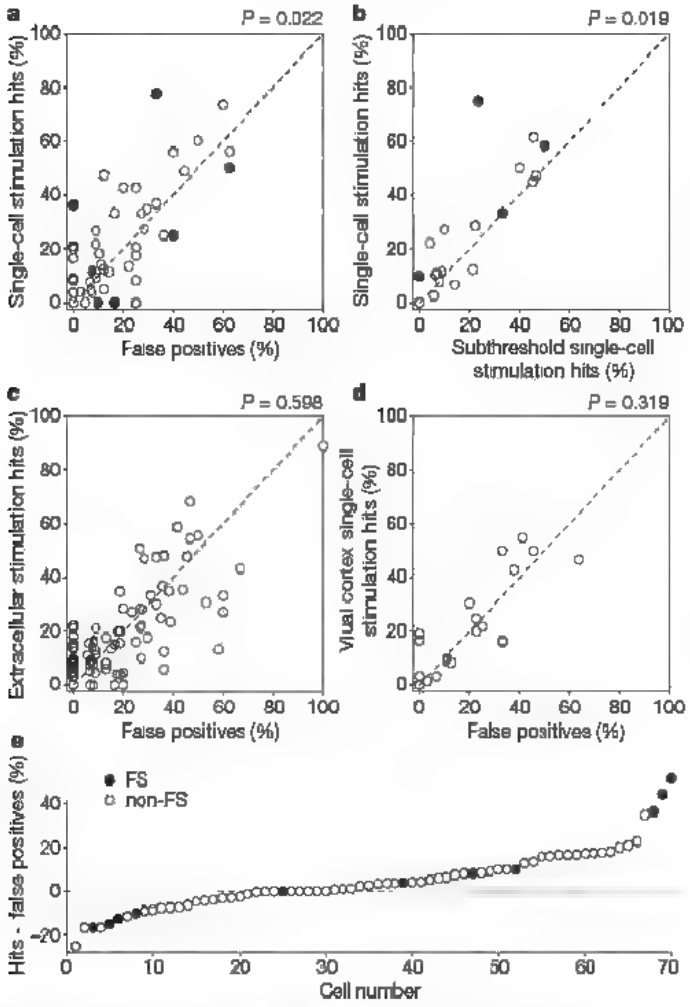


Figure 3 | Initiation of action potentials in single barrel cortex neurons causes biases towards respond ng. **a**, Response rates for single-cell stimulation trials (hits) versus no-current-injection catch trials (false positives) ($n = 51$ neurons; note several points coincide). Fast spiking, putative interneurons (filled circles), non fast spiking, putative excitatory neurons (empty circles). **b**, Response rates for single-cell stimulation trials (hits) versus subthreshold current injection catch trials ($n = 19$ neurons). Conventions as in **a**. **c**, Response rates for trials in which we applied 25 nA (twice the average juxtacellular stimulation current) into extracellular space (hits) versus no-current-injection catch trials (false positives) ($n = 90$ stimulation sites). **d**, Response rates for single-cell stimulation trials (hits) versus no-current-injection catch trials (false positives) for visual cortex neurons ($n = 21$), while microstimulation was applied in barrel cortex. Animals had been trained to report microstimulation in barrel cortex. **e**, Distribution of sensory effects (single-cell stimulation hit rate − catch trial response rate) across putative interneurons and putative excitatory neurons in barrel cortex single-cell stimulation experiments (conventions as in **a**).

Reaction times for single-cell stimulation were long and variable (Figs 2 and 4a, b) compared with microstimulation responses (Fig. 4c). Although effect size could be considerable in individual cells (Fig. 2), we did not observe single-cell responses with close to 100% hit rates and short reaction times as observed often in microstimulation trials. Thus, single cells never led to a strong, perceptually saturating signal. Microstimulation at 2–8 μA presumably activates multiple neurons, which may account for the difference between single-cell stimulation and microstimulation. Even smaller currents (less than 2 μA) are known to activate cortical neurons[16]. It remains to be seen if such currents lead to a microstimulation performance comparable to that of single-cell stimulation.

The combination of single-cell stimulation and control experiments shows that the activity of single sensory cortical neurons can lead to a behaviourally reportable effect. It has been estimated that a single barrel cortical column contains approximately 8,500 excitatory cells that generate about 1,550 spontaneous action potentials in a 200 ms period and about 4,000 action potentials in response to a small (3.3°) whisker deflection[17] that is close (about 60% hit rate) to the animal's detection threshold[18]. Given these numbers it is surprising that adding approximately 14 action potentials over 200 ms in a neuron is detectable. Other measurements suggest lower rates of

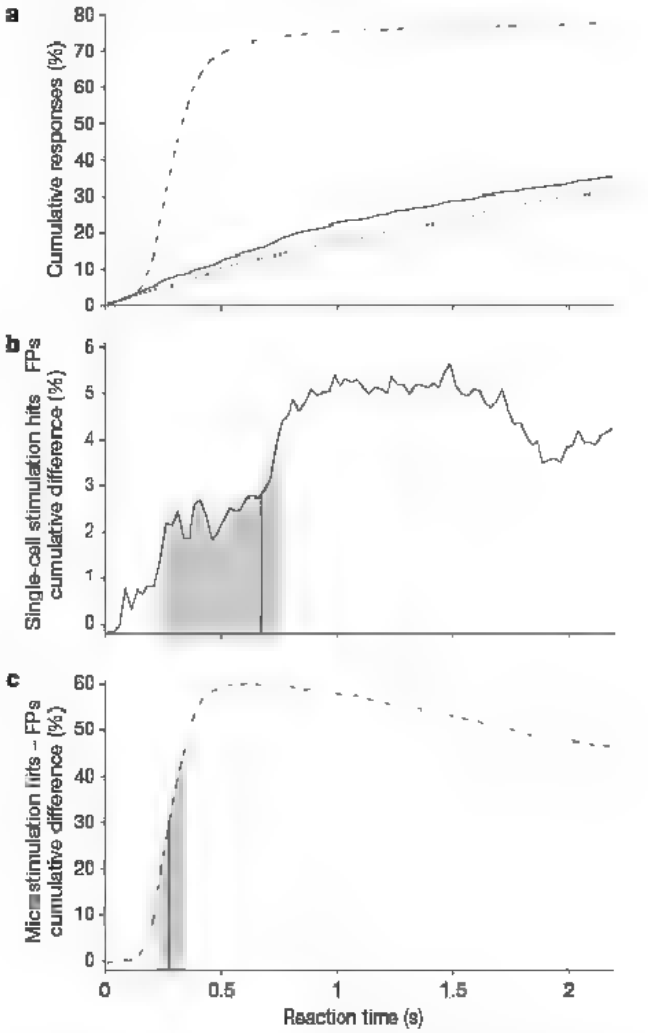


Figure 4 | Reaction times for single-cell stimulation are long and variable compared with microstimulation responses. **a**, Cumulative distribution of reaction times for microstimulation (dashed), single-cell stimulation (solid) and catch trials (dotted). **b**, Difference of the cumulative distributions of reaction times for single-cell stimulation and catch trials. This isolates the contribution of single-cell stimulation from false-positive responses. The vertical line marks the time where 50% of the peak difference is reached; the grey area marks the time from 25% to 75% of the peak difference. **c**, Difference of the cumulative distributions of reaction times for microstimulation and catch trials. Conventions as in **b**.

ongoing[19–21] and sensory-evoked[22–25] cortical action potential activity. The detectability of single-cell stimulation might therefore be related to the sparseness of cortical activity. A single cortical pyramidal cell connects to several thousand postsynaptic neurons[26], but most of these connections are weak[27]. Likewise, a single inhibitory neuron connects to thousands of local neurons. Depending on ongoing membrane potential fluctuations, variable sets of postsynaptic cells may become activated or suppressed, which might contribute to the variable reaction times. Some models of sensory decision making contain a temporal integration step during which sensory evidence is accumulated[28]. It is conceivable that the weak single-cell signals require longer temporal integration, thereby contributing to the long reaction times. The mechanisms that hold the sensory information between single-cell stimulation and reaction remain to be determined.

Our finding that stimulation of putative interneurons (which project locally) can lead to strong sensory effects suggests that (1) local circuits are involved in the read-out of single-cell activity and that (2) read-out mechanisms are sensitive to suppression of action potentials. Cortical microstimulation evokes pronounced and long-lasting inhibition (Fig. 2 and Supplementary Fig. 2). Thus, our animals might have been trained to detect the suppression of activity. The present results, with the classic single afferent stimulation experiments by Vallbo and colleagues[29] and single-cell stimulation experiments in rat motor cortex[30], demonstrate the behavioural relevance of single neuron activity. These studies establish a reverse physiology approach in which one analyses responses to cellular activity rather than cellular responses as in classical physiology. In further studies it should be possible to establish how the frequency and number of action potentials are related to the evoked sensations.

METHODS SUMMARY

Animals were trained to report microstimulation (40 cathodal pulses at 200 Hz, 0.3 ms pulse duration) applied to the barrel cortex through a tungsten microelectrode at a depth of 1,500 μm. In the first training session, current intensities no greater than 200 μA were applied; subsequently current intensity was decreased according to detection performance. During training animals were put on a water restriction schedule with daily access to water *ad libitum* for one hour after the experiment. Once the animal performed at detection thresholds no greater than 5 μA in at least one block of trials on two consecutive days, we switched to the single-cell stimulation report task; here microstimulation currents were on average 5.0 ± 1.6 μA. To stimulate single neurons close to the microstimulation site, we glued a tungsten microelectrode close to the tip of a glass pipette (average tip separation approximately 75 μm). The construct was inserted through the intact dura to a mean depth of 1,400 ± 271 μm, whereby the actual depth from the cortical surface was less because of dimpling and oblique penetrations. From the histologically identified neurons it appears that most single-cell stimulation experiments were performed in cortical layers 4, 5A and 5B. Cells were classified as fast spiking neurons (putative interneurons) if the action potential width was no greater than 0.4 ms (peak to trough) and/or if they responded with at least 50 action potentials (that is, at least 250 Hz) during at least one 200 ms current injection (see Supplementary Fig. 8). The remainder of cells were classified as non-fast spiking (putative excitatory) cells.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 7 July; accepted 1 November 2007.
Published online 19 December 2007.

1. Rieke, F., Warland, D., de Ruyter Van Steveninck, R. R. & Bialek, W. *Spikes: Exploring the Neural Code* (MIT Press, Cambridge, Massachusetts, 1997).
2. Britten, K. H., Newsome, W. T., Shadlen, M. N., Celebrini, S. & Movshon, J. A. A relationship between behavioral choice and the visual responses of neurons in macaque MT. *Vis. Neurosci.* 13, 87–100 (1996).
3. Afraz, S. R., Kiani, R. & Esteky, H. Microstimulation of inferotemporal cortex influences face categorization. *Nature* 442, 692–695 (2006).
4. Salzman, C. D., Britten, K. H. & Newsome, W. T. Cortical microstimulation influences perceptual judgements of motion direction. *Nature* 346, 174–177 (1990).
5. Murasugi, C. M., Salzman, C. D. & Newsome, W. T. Microstimulation in visual area MT: effects of varying pulse amplitude and frequency. *J. Neurosci.* 13, 1719–1729 (1993).
6. Tehovnik, E. J. Electrical stimulation of neural tissue to evoke behavioral responses. *J. Neurosci. Methods* 65, 1–17 (1996).
7. Zilles, K. & Wree, A. in *The Rat Nervous System* (ed. Paxinos, G.) 649–685 (Academic Press, London, 1995).
8. Beaulieu, C. Numerical data on neocortical neurons in adult rat, with special reference to the GABA population. *Brain Res.* 609, 284–292 (1993).
9. Penfield, W. & Rasmussen, T. *The Cerebral Cortex of Man: A Clinical Study of Localization of Function* (Macmillan, New York, 1950).
10. Romo, R., Hernandez, A., Zainos, A. & Salinas, E. Somatosensory discrimination based on cortical microstimulation. *Nature* 392, 387–390 (1998).
11. Krauss, A., Manns, I. D. & Brecht, M. in *Annu. Meeting Soc. Neurosci.* 58.19 (2003).
12. Schmidt, E. M. *et al.* Feasibility of a visual prosthesis for the blind based on intracortical microstimulation of the visual cortex. *Brain* 119, 507–522 (1996).
13. Bartlett, J. R. & Doty, R. W. An exploration of the ability of macaques to detect microstimulation of striate cortex. *Acta Neurobiol. Exp. (Wars.)* 40, 713–727 (1980).
14. Pinault, D. A novel single-cell staining procedure performed *in vivo* under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin. *J. Neurosci. Methods* 65, 113–136 (1996).
15. Talwar, S. K. & Gerstein, G. L. Auditory frequency discrimination in the white rat. *Hear. Res.* 126, 135–150 (1998).
16. Swadlow, H. A. Monitoring the excitability of neocortical efferent neurons to direct activation by extracellular current pulses. *J. Neurophysiol.* 68, 605–619 (1992).
17. de Kock, C. P., Bruno, R. M., Spors, H. & Sakmann, B. Layer- and cell-type-specific suprathreshold stimulus representation in rat primary somatosensory cortex. *J. Physiol. (Lond.)* 581, 139–154 (2007).
18. Stuttgen, M. C., Ruter, J. & Schwarz, C. Two psychophysical channels of whisker deflection in rats align with two neuronal classes of primary afferents. *J. Neurosci.* 26, 7933–7941 (2006).
19. Kerr, J. N., Greenberg, D. & Helmchen, F. Imaging input and output of neocortical networks *in vivo*. *Proc. Natl Acad. Sci. USA* 102, 14063–14068 (2005).
20. Margrie, T. W., Brecht, M. & Sakmann, B. *In vivo*, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. *Pflugers Arch.* 444, 491–498 (2002).
21. Waters, J. & Helmchen, F. Background synaptic activity is sparse in neocortex. *J. Neurosci.* 26, 8267–8277 (2006).
22. Brecht, M. & Sakmann, B. Dynamic representation of whisker deflection by synaptic potentials in spiny stellate and pyramidal cells in the barrels and septa of layer 4 rat somatosensory cortex. *J. Physiol. (Lond.)* 543, 49–70 (2002).
23. Moore, C. I. & Nelson, S. B. Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. *J. Neurophysiol.* 80, 2882–2892 (1998).
24. Olshausen, B. A. & Field, D. J. Sparse coding of sensory inputs. *Curr. Opin. Neurobiol.* 14, 481–487 (2004).
25. Warren, R. A. & Dykes, R. W. Population analysis of single neurons in cat somatosensory cortex. *Somatosens. Mot. Res.* 9, 297–312 (1992).
26. DeFelipe, J. & Farinas, I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog. Neurobiol.* 39, 563–607 (1992).
27. Song, S., Sjostrom, P. J., Reigl, M., Nelson, S. & Chklovskii, D. B. Highly nonrandom features of synaptic connectivity in local cortical circuits. *PLoS Biol.* 3, e68 (2005).
28. Gold, J. I. & Shadlen, M. N. Representation of a perceptual decision in developing oculomotor commands. *Nature* 404, 390–394 (2000).
29. Vallbo, A. B., Olsson, K. A., Westberg, K. G. & Clark, F. J. Microstimulation of single tactile afferents from the human hand. Sensory attributes related to unit type and properties of receptive fields. *Brain* 107, 727–749 (1984).
30. Brecht, M., Schneider, M., Sakmann, B. & Margrie, T. W. Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex. *Nature* 427, 704–710 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank B. Sakmann for suggesting the juxtacellular stimulation approach, J. van der Burg, E. Haasdijk and G. Maas for technical contributions, P. den Ieger and A. Lee for discussions, and G. Borst, M. Frens, C. Hansel, L. Herfst, A. Lee, B. Voigt and J. Wolfe for comments on the manuscript. This work was supported by the Bernstein Center for Computational Neuroscience and Humboldt University Berlin, Erasmus MC, and VIDI (NWO) and HFSP grants to M.B.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.R.H. (arthur.houweling@bccn-berlin.de) or M.B. (michael.brecht@bccn-berlin.de).

LETTERS

TRPC channel activation by extracellular thioredoxin

Shang-Zhong Xu[1]*†, Piruthivi Sukumar[1]*, Fanning Zeng[1], Jing Li[1], Amit Jairaman[1], Anne English[3], Jacqueline Naylor[1], Coziana Ciurtin[1], Yasser Majeed[1], Carol J. Milligan[1], Yahya M. Bahnasi[1], Eman Al-Shawaf[1], Karen E. Porter[2], Lin-Hua Jiang[1], Paul Emery[3], Asipu Sivaprasadarao[1] & David J. Beech[1]

Mammalian homologues of *Drosophila melanogaster* transient receptor potential (TRP) are a large family of multimeric cation channels that act, or putatively act, as sensors of one or more chemical factor[1,2]. Major research objectives are the identification of endogenous activators and the determination of cellular and tissue functions of these channels. Here we show the activation of TRPC5 (canonical TRP 5) homomultimeric and TRPC5–TRPC1 heteromultimeric channels[3–5] by extracellular reduced thioredoxin, which acts by breaking a disulphide bridge in the predicted extracellular loop adjacent to the ion-selectivity filter of TRPC5. Thioredoxin is an endogenous redox protein with established intracellular functions, but it is also secreted and its extracellular targets are largely unknown[6–9]. Particularly high extracellular concentrations of thioredoxin are apparent in rheumatoid arthritis[8,10–12], an inflammatory joint disease that disables millions of people worldwide[13]. We show that TRPC5 and TRPC1 are expressed in secretory fibroblast-like synoviocytes from patients with rheumatoid arthritis, that endogenous TRPC5–TRPC1 channels of the cells are activated by reduced thioredoxin, and that blockade of the channels enhances secretory activity and prevents the suppression of secretion by thioredoxin. The data indicate the presence of a previously unrecognized ion-channel activation mechanism that couples extracellular thioredoxin to cell function.

TRPC5 is markedly activated by extracellular lanthanide ions[4,14,15]. The effects of these ions depend on a glutamic acid residue at position 543 (ref. 14) in the predicted extracellular loop adjacent to the ion pore (Supplementary Figs 1 and 2). This structural feature may therefore have functional importance in enabling extracellular factors to activate the channels. Because lanthanides are unlikely to be physiological activators, we were interested in alternatives and developed a hypothesis based on amino acid sequence alignment, which showed two cysteine residues near glutamic acid 543 that are conserved in TRPC5, TRPC4 and TRPC1 (Supplementary Fig. 2), a subset of the seven TRPC channels[1–5]. TRPC5 and TRPC4 have similar functional properties[4] and both form heteromultimers with TRPC1 (refs 3–5), a subunit that has weak targeting to the plasma membrane when expressed in isolation[3,16].

Pairs of cysteine residues may be covalently linked by a disulphide bridge that can be cleaved by reduction. We therefore applied the chemical reducing agent dithiothreitol (DTT) to HEK-293 cells expressing TRPC5 (refs 15, 16). There was channel activation with the characteristic current–voltage (*I–V*) relationship of TRPC5 and blocking by 2-aminoethoxydiphenyl borate (2-APB), an inhibitor of TRPC5 (ref. 5) (Fig. 1a, b, d). Current recovered on wash-out of DTT (data not shown). Similarly, the membrane-impermeable disulphide reducing agent Tris (2-carboxyethyl) phosphine hydrochloride (TCEP; Fig. 1c, d) activated TRPC5, whereas the thiol reagent [2-(trimethylammonium) ethyl]methanethiosulphonate bromide (MTSET) had no effect (Fig. 1d). TRPC5 was inhibited by cadmium ions only after pretreatment with DTT (Fig. 1e, f), which is consistent

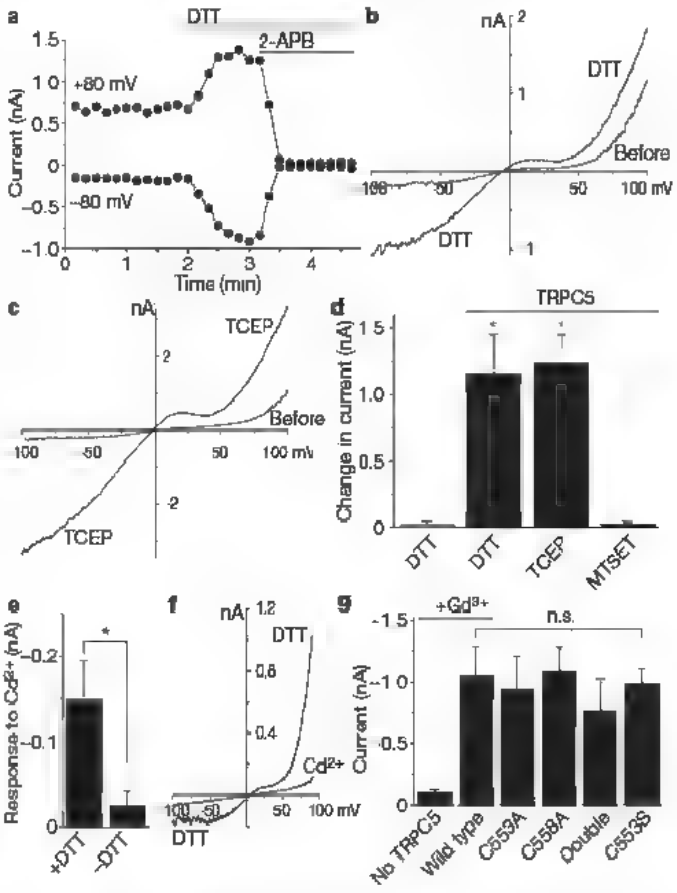


Figure 1 | Functional disulphide bridge in TRPC5. Whole-cell recordings from HEK-293 cells **a**, In a cell expressing TRPC5, response to bath-applied 10 mM DTT and 75 μM 2-APB. **b**, *I–V* relationship from **a**. **c**, As for **b** but with 1 mM TCEP. **d**, Currents at −80 mV evoked by 10 mM DTT ($n = 8$), 1 mM TCEP ($n = 5$) or 5 mM MTSET ($n = 6$) in cells expressing TRPC5. DTT had no effect without TRPC5 ($n = 5$). **e**, Inhibition of current at −80 mV by 0.1 mM Cd^{2+} in TRPC5-expressing cells with and without DTT treatment. **f**, As for **e** but typical *I–V* relationships. **g**, Currents at −80 mV after transfection with green fluorescent protein (GFP) alone (no TRPC5, $n = 8$) or GFP plus wild-type TRPC5 ($n = 7$) or the TRPC5 mutants C553A ($n = 11$), C558A ($n = 6$), C553A + C558A (double, $n = 3$) or C553S ($n = 6$). Gd^{3+} (100 μM) activated wild-type TRPC5 but had no effect on mutants. All currents were blocked by 2-APB (see, for example, Supplementary Fig. 5). Where error bars are shown, results are expressed as means and s.e.m. Asterisk, $P < 0.05$; n.s., no significant difference.

[1]Institute of Membrane and Systems Biology, Garstang Building, Faculty of Biological Sciences, and [2]School of Medicine, University of Leeds, Leeds LS2 9JT, UK. [3]Academic Unit of Musculoskeletal Disease, Chapel Allerton Hospital, Leeds LS7 4SA, UK. †Present address: Postgraduate Medical Institute & Hull York Medical School, University of Hull, Cottingham Road, Hull HU6 7RX, UK.
*These authors contributed equally to this work

with the metal ions acting by re-engaging cysteine residues[17]. Other TRP channels lacking the cysteine pair in a similar position were unresponsive to DTT (Supplementary Figs 2 and 3). The data support the hypothesis that the cysteine pair in TRPC5 normally engages in a disulphide bridge that constrains the channel in a state of limited opening probability, enabling enhanced channel activity when the bridge is broken.

To test the hypothesis further, we expressed TRPC5 mutants containing alanine in place of cysteine. Such mutants were constitutively active and were not stimulated by reducing agent or lanthanide (Fig. 1g and Supplementary Figs 4 and 5). Ionic currents for the single mutants (C553A, C553S or C558A) and double mutant (C553A + C558A) were not significantly different, suggesting that the two cysteine residues have a joint role (Fig. 1g). Expression of wild-type TRPC1 together with the TRPC5 double mutant led to smaller constitutive currents that were not affected by DTT or lanthanide, which is consistent with TRPC1 suppressing the current amplitude but not conferring a functional effect of reducing agents (Supplementary Fig. 6). Dimers of TRPC5 were not detected under non-reducing conditions, suggesting an intra-subunit rather than inter-subunit disulphide bridge (Supplementary Fig. 7).

Thioredoxin is an important redox protein with established biological roles including those in cancer, ischaemic reperfusion injury, inflammation and ageing[8]. It is both an intracellular and secreted protein[6–9]. It is reduced by the NADPH-dependent flavoprotein thioredoxin reductase and in this form has the capability of breaking disulphide bridges[8]. Extracellular reduced thioredoxin (rTRX) acts similarly to DTT, causing TRPC5 activation (Fig. 2a, b). We therefore proposed that rTRX is a previously unrecognized endogenous extracellular regulator of TRPC5. In taking this idea forward we also considered TRPC1 because many cells endogenously expressed TRPC5 and TRPC1 together, leading to TRPC5–TRPC1 heteromultimers[3,5,16,18]. The TRPC5–TRPC1 channel is also activated by rTRX or DTT (Fig. 2c). Consistent with previous reports[3,16] was our observation that the TRPC5 and TRPC5–TRPC1 channels had distinct 'fingerprint' *I–V* relationships (for example Fig. 3b and Supplementary Fig. 14).

Thioredoxin concentrations up to a mean of 0.41 μg ml^{-1} (maximum 1.2 μg ml^{-1}) have been detected in serum and synovial fluid from patients with rheumatoid arthritis[8,10–12]. Furthermore, reducing capability of thioredoxin exists in serum; thioredoxin reductase occurs in human joints and its activity is correlated with disease

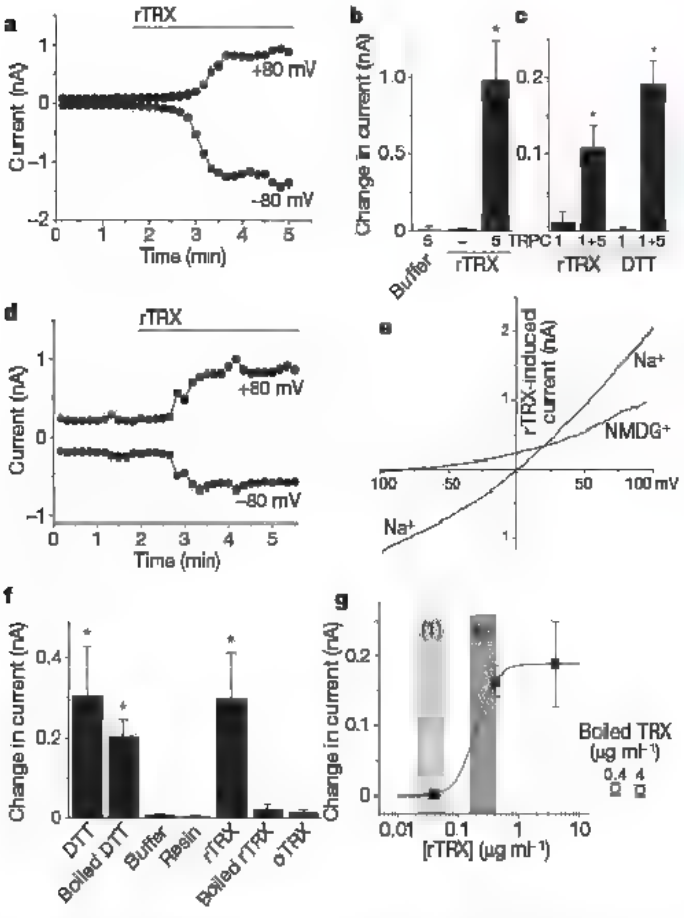


Figure 2 | Ionic current induced by rTRX. **a–c**, Whole-cell current data from HEK-293 cells expressing TRPC5 alone (**a**, **b**), TRPC1 alone or TRPC5 plus TRPC1 (**c**) **a**, Effect of 4 μg ml^{-1} rTRX. **b**, Current at −80 mV in response to elution buffer diluted 1:100 ($n = 4$) or rTRX with ($n = 8$) and without ($n = 3$) TRPC5 expression. **c**, Responses to rTRX or 10 mM DTT ($n = 5$ for each) **d**, Effect of rTRX on a human FLS cell. **e–g**, Data for rabbit FLS cells. **e**, rTRX induced *I–V* relationships in standard bath (Na^+) or *N*-methyl-D-glucamine ($NMDG^+$) solution (see Supplementary Fig. 9) **f**, Currents evoked at −80 mV **g**, Data for human rTRX with a fitted Hill equation (concentration giving half-maximal response 0.20 μg ml^{-1}, slope 2.64) Open symbols are control data and shaded areas are the concentrations of TRX in patients without arthritis (1) or with osteoarthritis (1) or rheumatoid arthritis (2) In **f** and **g**, $n = 5$ per data point Where error bars are shown, results are expressed as means and s.e.m. Asterisk, $P < 0.05$.

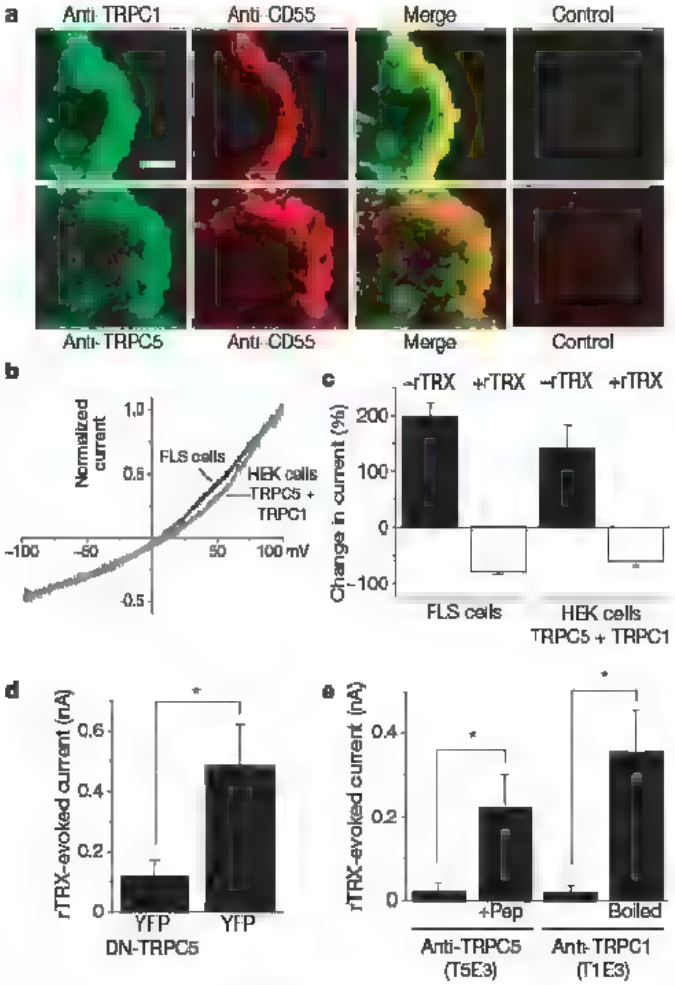


Figure 3 | Endogenous TRPC expression and function. **a**, Tissue sections from joints of patients with rheumatoid arthritis stained with T1E3 or T5E3 (green) or anti-CD55 (red) antibodies. Controls were omission of anti-CD55 antibody, and T1E3 or T5E3 preadsorbed on its antigenic peptide. **b**, Normalized rTRX-evoked *I–V* relationships for rabbit FLS cells ($n = 3$) and HEK-293 cells expressing TRPC5 and TRPC1 ($n = 5$). **c**, Changes in currents at −80 mV in response to 10 μM La^{3+} before or after treatment with 4 μg ml^{-1} rTRX (FLS cells, $n = 6$; HEK cells, $n = 4$) **d**, Current at −80 mV in FLS cells transfected with dominant-negative (DN) TRPC5 plus yellow fluorescent protein (YFP) or YFP alone ($n = 5$ for each) **e**, As in **d** but showing the effects of anti-TRPC antibodies ($n = 5$ for each). Pep, antigenic peptide. Where error bars are shown, results are expressed as means and s.e.m. Asterisk, $P < 0.05$.

severity[16,19,20] (see also Supplementary Results and Supplementary Discussion). We therefore considered whether the activation of TRPC5 by rTRX is relevant to the cells that secrete synovial fluid, the CD55-positive fibroblast-like synoviocytes (FLS cells). CD55-positive FLS cells (Supplementary Fig. 8) showed a non-selective cationic current in response to DTT or rTRX (Fig. 2d–g and Supplementary Figs 9 and 10). The mean current evoked by rTRX at −80 mV in FLS cells from the knee joint of patients with rheumatoid arthritis was -0.85 ± 0.42 nA (mean ± s.e.m.; $n = 14$). Oxidized TRX (oTRX) had no effect (Fig. 2f). The effective concentrations of rTRX indicate a possible relevance to rheumatoid arthritis (Fig. 2g). Nitric oxide is an alternative endogenous regulator of cysteine residues[21]; however, it failed to evoke current in FLS cells, even at a concentration 100-fold that required to evoke vasorelaxation (Supplementary Fig. 10, Supplementary Results and Supplementary Discussion).

There have been no previous reports on the expression of TRPC channels in synovial joints, so we explored synovial tissue biopsies from patients with rheumatoid arthritis. TRPC5 and TRPC1 proteins were detected and localized together with CD55 (Fig. 3a). Similarly, the FLS cells used in our electrophysiological experiments expressed messenger RNAs encoding TRPC5 and TRPC1, western blotting indicated the presence of TRPC5 and TRPC1 proteins, and immunolabelling revealed TRPC5 and TRPC1 at the cell surface (Supplementary Figs 8, 11 and 13).

The *I–V* relationship of the rTRX-evoked current in FLS cells was similar to that of the TRPC5–TRPC1 heteromultimeric channel (Fig. 3b). Furthermore, experiments with lanthanum ions showed unusual and striking similarity between the endogenous current and the current of overexpressed TRPC5–TRPC1: in the absence of a reducing agent, lanthanum ions stimulated current in both HEK-293 cells (exogenously expressing TRPC5–TRPC1) and FLS cells, whereas after the induction of current by rTRX, lanthanum ions were inhibitory in both cases (Fig. 3c and Supplementary Fig. 9). Also consistent with the involvement of TRPC channels were the observations that the rTRX-evoked current of FLS cells was blocked by 2-APB and that the inward current was suppressed when most of the extracellular Na^+ was replaced by the bulky and impermeant cation *N*-methyl-D-glucamine (Fig. 2e and Supplementary Fig. 9). As a further test of the involvement of TRPC5 and TRPC1, FLS cells were transfected with a dominant-negative ion-pore mutant of TRPC5 that inhibited native channels capable of interacting with TRPC5 (refs 16, 22). The mutant suppressed current evoked by rTRX (Fig. 3d).

Further evidence that TRPC5 and TRPC1 contribute to the endogenous rTRX-responsive channel of FLS cells came from studies with anti-TRPC5 (T5E3) and anti-TRPC1 (T1E3) antibodies, which target the predicted extracellular loop region and specifically block the functions of TRPC5 and TRPC1, respectively[23–25]. T5E3 and T1E3 antibodies labelled unpermeabilized FLS cells, unlike antibody targeted to the intracellular carboxy terminus of TRPC5, which labelled only permeabilized cells (Supplementary Figs 8 and 11), indicating that TRPC5 and TRPC1 are transmembrane proteins with extracellular epitopes. Like dominant-negative mutant TRPC5, T5E3 or T1E3 suppressed rTRX-evoked current (Fig. 3e). Antibody targeted to CD55, which is a membrane protein unrelated to TRP, had no significant effect ($n = 7$; data not shown). Gene expression, electrophysiology, pharmacology, recombinant DNA and antibody studies therefore yielded data consistent with the carrying of rTRX-evoked current in FLS cells by a channel containing TRPC5 and TRPC1.

One of the functions of FLS cells is to secrete matrix metalloproteinases (MMPs), which are associated with tissue remodelling and the progression of arthritis[26]. The use of zymography to detect gelatinase activities of MMP-2 and MMP-9 secreted from rabbit FLS cells (Supplementary Fig. 12) revealed that T5E3 and T1E3 antibodies have large stimulatory effects (Fig. 4a, b). Human FLS cells showed greater MMP-2 secretion than that of MMP-9 (compare Supplementary Fig. 12 with Fig. 4a). Enzyme-linked immunosorbent assays (ELISAs) for human MMP-2 enabled the quantification of the absolute concentration of total MMP-2 secreted; again, either T5E3 or T1E3 antibody had a profound stimulatory effect (Fig. 4c). Similarly, knockdown of expression of the genes encoding TRPC1 and TRPC5 by RNA-mediated interference enhanced the secretion of MMP-2 (Supplementary Fig. 13). Inhibition of MMP secretion by the addition of exogenous reducing TRX was lost in the presence of T5E3 (Fig. 4d). Similar data were obtained for pro-MMP-1 secretion from human FLS cells (Fig. 4e, f) and MMP-9 measured by zymography in rabbit FLS cells ($n = 6$; data not shown). The data therefore reveal constitutive and rTRX-evoked activity of TRPC5 and TRPC1 channels that inhibits the secretion of MMP from FLS cells.

The data of this study indicate that secreted TRX is a type of ion channel agonist that acts through its reduced form to break a restraining intra-subunit disulphide bridge between cysteine residues in TRPC5, thereby stimulating the channel either as a homomeric assembly or as a heteromultimer with TRPC1. A transduction mechanism is therefore revealed that can directly couple cell activity to extracellular reduced thioredoxin. This mechanism may have particular relevance in conditions such as rheumatoid arthritis, in which TRX concentrations are strongly elevated, but the broad distributions of TRX and the channels indicate that the mechanism could be widely used.

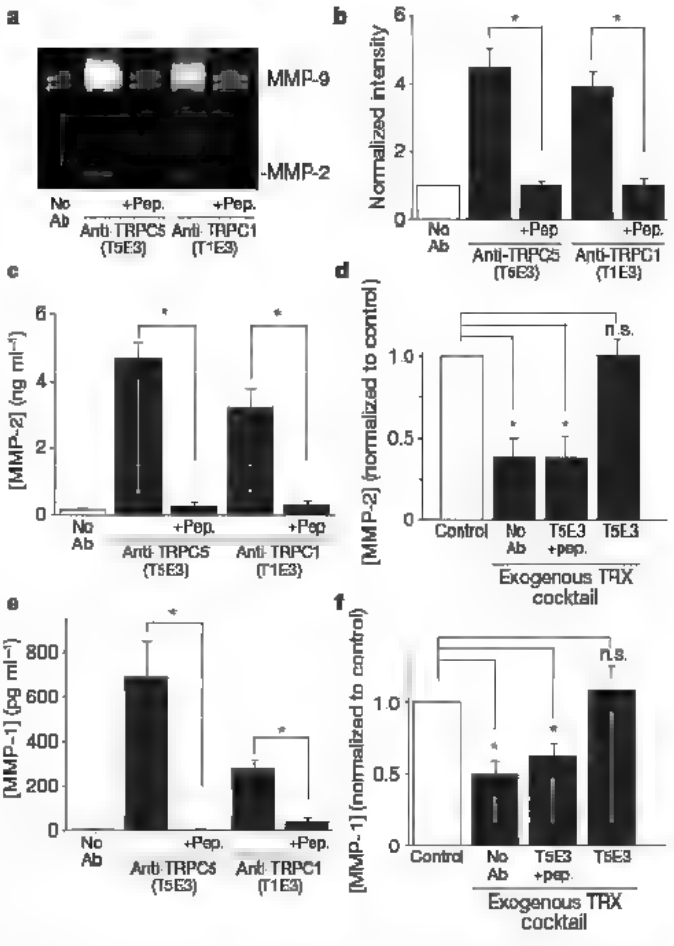


Figure 4 | Relevance to secretion from FLS cells. **a**, Zymogram showing MMP-9 (pro and active) and MMP-2 from rabbit. Ab, antibody **b**, As for **a** but mean data after normalization of rabbit MMP-9 band intensity to the control group without antibody ($n = 3$ for each) **c**, ELISA data for human MMP-2 ($n = 4$) **d**, Effect of T5E3 ($n = 4$) on inhibition of human MMP-2 secretion by exogenous TRX cocktail. For each group, secretion in TRX was normalized to that in its absence (control). **e, f**, As for **c, d**, but for secretion of human pro-MMP-1. Results are expressed as means and s.e.m. Asterisk, $P < 0.05$; n.s., no significant difference.

METHODS SUMMARY

Cells. Synovial tissue biopsies were obtained with informed consent from patients diagnosed with rheumatoid arthritis at the Academic Unit of Musculoskeletal Disease, Chapel Allerton Hospital, Leeds. Ethical approval was given by the local ethics committee. Human synovial tissue biopsies were washed with PBS and digested in 0.25% type IA collagenase for 4 h at 37 °C, after which FLS cells were cultured in DMEM/F-12 + Glutamax (Gibco). HEK 293 cells were grown in DMEM-F12 (Gibco) and rabbit FLS cells (HIG82; ATCC) were grown in Ham's F12 (Gibco). Culture media contained 10% fetal calf serum, 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air and replated on coverslips or 24-well plates before experiments.

Electrophysiology. Whole-cell patch-clamp recordings were performed[15,16] at 21 ± 2 °C using patch pipette solution containing (in mM): 115 CsCl, 10 EGTA, 2 $MgCl_2$, 5 Na_2ATP, 0.1 NaGTP, 10 HEPES, 5.7 $CaCl_2$; the pH was adjusted to 7.2 with CsOH. The standard bath solution contained (in mM): 130 NaCl, 5 KCl, 8 D-glucose, 10 HEPES, 1.2 $MgCl_2$ and 1.5 $CaCl_2$; the pH was adjusted to 7.4 with NaOH.

Data analysis. Ionic currents are shown as positive values when they increased in response to a treatment, and as negative values when they decreased. Data are expressed as means and s.e.m., where *n* is the number of individual experiments. Data sets were compared by using paired or unpaired Student's *t*-tests, with a significant difference indicated by $P < 0.05$ (asterisk) and no difference by n.s. All human tissue or cell data are derived from, or are representative of, at least three independent experiments on samples from three patients.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 6 July; accepted 24 October 2007.

1. Flockerzi, V. An introduction on TRP channels. *Handb. Exp. Pharmacol.* 179, 1–19 (2007).
2. Nilius, B., Owsianik, G., Voets, T. & Peters, J. A. Transient receptor potential cation channels in disease. *Physiol. Rev.* 87, 165–217 (2007).
3. Strübing, C., Krapivinsky, G., Krapivinsky, L. & Clapham, D. E. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron* 29, 645–655 (2001).
4. Plant, T. D. & Schaefer, M. TRPC4 and TRPC5: receptor-operated Ca^{2+}-permeable nonselective cation channels. *Cell Calcium* 33, 441–450 (2003).
5. Beech, D. J. Canonical transient receptor potential 5. *Handb. Exp. Pharmacol.* 179, 109–123 (2007).
6. Rubartelli, A., Bajetto, A., Allavena, G., Wollman, E. & Sitia, R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J. Biol. Chem.* 267, 24161–24164 (1992).
7. Arner, E. S. & Holmgren, A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem.* 267, 6102–6109 (2000).
8. Burke-Gaffney, A., Callister, M. E. & Nakamura, H. Thioredoxin: friend or foe in human disease? *Trends Pharmacol. Sci.* 26, 398–404 (2005).
9. Schwertassek, U. *et al.* Selective redox regulation of cytokine receptor signaling by extracellular thioredoxin-1. *EMBO J.* 26, 3086–3097 (2007).
10. Maurice, M. M. *et al.* Expression of the thioredoxin–thioredoxin reductase system in the inflamed joints of patients with rheumatoid arthritis. *Arthritis Rheum.* 42, 2430–2439 (1999).
11. Yoshida, S. *et al.* Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-α-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. *J. Immunol.* 163, 351–358 (1999).
12. Jikimoto, T. *et al.* Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis. *Mol. Immunol.* 38, 765–772 (2002).
13. Smolen, J. S., Aletaha, D., Koeller, M., Weisman, M. H. & Emery, P. New therapies for treatment of rheumatoid arthritis. *Lancet* advance online publication doi:10.1016/S0140-6736(07)60784-3 (13 June 2007).
14. Jung, S. *et al.* Lanthanides potentiate TRPC5 currents by an action at extracellular sites close to the pore mouth. *J. Biol. Chem.* 278, 3562–3571 (2003).
15. Zeng, F. *et al.* Human TRPC5 channel activated by a multiplicity of signals in a single cell. *J. Physiol. (Lond.)* 559, 739–750 (2004).
16. Xu, S. Z. *et al.* A sphingosine-1-phosphate-activated calcium channel controlling vascular smooth muscle cell motility. *Circ. Res.* 98, 1381–1389 (2006).
17. Elliott, D. J. *et al.* Molecular mechanism of voltage sensor movements in a potassium channel. *EMBO J.* 23, 4717–4726 (2004).
18. Riccio, A. *et al.* mRNA distribution analysis of human TRPC family in CNS and peripheral tissues. *Brain Res. Mol. Brain Res.* 109, 95–104 (2002).
19. Lemarechal, H. *et al.* High redox thioredoxin but low thioredoxin reductase activities in the serum of patients with rheumatoid arthritis. *Clin. Chim. Acta* 367, 156–161 (2006).
20. Lemarechal, H. *et al.* Impairment of thioredoxin reductase activity by oxidative stress in human rheumatoid synoviocytes. *Free Radic. Res.* 41, 688–698 (2007).
21. Yoshida, T. *et al.* Nitric oxide activates TRP channels by cysteine S-nitrosylation. *Nature Chem. Biol.* 2, 596–607 (2006).
22. Strübing, C., Krapivinsky, G., Krapivinsky, L. & Clapham, D. E. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J. Biol. Chem.* 278, 39014–39019 (2003).
23. Xu, S. Z. & Beech, D. J. TrpC1 is a membrane-spanning subunit of store-operated Ca^{2+} channels in native vascular smooth muscle cells. *Circ. Res.* 88, 84–87 (2001).
24. Xu, S. Z. *et al.* Generation of functional ion-channel tools by E3 targeting. *Nature Biotechnol.* 23, 1289–1293 (2005).
25. Xu, S. Z., Boulay, G., Flemming, R. & Beech, D. J. E3-targeted anti-TRPC5 antibody inhibits store-operated calcium entry in freshly isolated pial arterioles. *Am. J. Physiol. Heart Circ. Physiol.* 291, H2653–H2659 (2006).
26. Burrage, P. S., Mix, K. S. & Brinckerhoff, C. E. Matrix metalloproteinases: role in arthritis. *Front. Biosci.* 11, 529–543 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements. This work was supported by Wellcome Trust grants to D.J.B. and A.S., and a Physiological Society Junior Fellowship to C.C. P.S. has an Overseas Research Scholarship and University Studentship, J.N. has a Biotechnology and Biological Sciences Research Council PhD studentship, Y.M. a university studentship and Y.B. a scholarship from the Egyptian Ministry of Higher Education.

Author Information. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to D.J.B. (d.j.beech@leeds.ac.uk).

LETTERS

Trisomy represses Apc^{Min}-mediated tumours in mouse models of Down's syndrome

Thomas E. Sussan[1]†, Annan Yang[1], Fu Li[2], Michael C. Ostrowski[2] & Roger H. Reeves[1]

Epidemiological studies spanning more than 50 yr reach conflicting conclusions as to whether there is a lower incidence of solid tumours in people with trisomy 21 (Down's syndrome)[1,2]. We used mouse models of Down's syndrome and of cancer in a biological approach to investigate the relationship between trisomy and the incidence of intestinal tumours. Apc^{Min}-mediated tumour number was determined in aneuploid mouse models Ts65Dn, Ts1Rhr and Ms1Rhr. Trisomy for orthologues of about half of the genes on chromosome 21 (Hsa21) in Ts65Dn mice or just 33 of these genes in Ts1Rhr mice resulted in a significant reduction in the number of intestinal tumours. In Ms1Rhr, segmental monosomy for the same 33 genes that are triplicated in Ts1Rhr resulted in an increased number of tumours. Further studies demonstrated that the *Ets2* gene contributed most of the dosage-sensitive effect on intestinal tumour number. The action of *Ets2* as a repressor when it is overexpressed differs from tumour suppression, which requires normal gene function to prevent cellular transformation. Upregulation of *Ets2* and, potentially, other genes involved in this kind of protective effect may provide a prophylactic effect in all individuals, regardless of ploidy.

The most widely used model of Down's syndrome is the Ts65Dn mouse, which is trisomic for orthologues of about 100 Hsa21 genes and recapitulates in detail several phenotypes of Down's syndrome[3,4] (Supplementary Fig. 1). Mice that are heterozygous for the Apc^{Min} mutation accumulate tumours analogous to those in familial adenomatous polyposis along the wall of the small intestine and colon[5]. *APC* is also mutated in a high proportion of spontaneous intestinal cancers in human beings. Although the mouse mutation is completely penetrant, the number of tumours that develop is dependent on both genetic modifier genes and environmental factors[6].

Female Ts65Dn mice were crossed to Apc^{Min} males and the number of tumours in the small intestine was determined in their trisomic and euploid Apc^{Min} progeny at 120 days of age (Supplementary Fig. 2). Trisomic mice showed a significant 44% reduction in the number of tumours compared to their euploid, Apc^{Min} littermates, from 45.4 to 23.8 tumours (Table 1). This establishes a biological basis for the effects of trisomy on tumour formation and shows that trisomy for orthologues of about half of the genes on Hsa21 is sufficient to reduce tumour incidence in this model.

We reanalysed these data considering the inheritance of susceptible or resistant alleles of the modifier of *Min 1* (*Mom1*) locus that result in higher or lower tumour number ($Mom1^s$ and $Mom1^r$, respectively; genetic background of all crosses is shown in Supplementary Fig. 3)[7,8]. The inheritance of a single $Mom1^r$ allele reduced the average tumour number from 62.6 to 21.3 in euploid mice (66%) as expected, and a similar 59% reduction occurred in Ts65Dn (Table 1). Ts65Dn, $Mom1^{s/s}$ mice had a highly significant 50% reduction in small intestine tumour number compared to euploid $Mom1^{s/s}$ mice ($P = 0.0028$). Trisomic mice that inherited a $Mom1^r$ allele ($Mom1^{s/r}$) also had substantially reduced tumour numbers relative to euploid mice, although this reduction did not reach a statistically significant level in the small sample of Ts65Dn, $Mom1^{s/r}$ mice available for this post-hoc analysis. Thus the $Mom1^r$ effect seems to be additive with the protective effect of trisomy, suggesting that independent mechanisms are involved.

We analysed Ts1Rhr mice to narrow the candidate region for the gene or genes responsible for reduced tumour number. These mice have segmental trisomy for 33 of the genes that are triplicated in Ts65Dn (Supplementary Fig. 1). These genes represent a 'critical region' of Hsa21, previously thought to be sufficient to cause several phenotypes of Down's syndrome[9]. Ts1Rhr, Apc^{Min} mice had a significant 26% reduction in the average number of tumours in the small intestine when compared to euploid, Apc^{Min} mice (Table 1).

When Apc^{Min} mice were crossed to Ms1Rhr, which have segmental monosomy for the 33 genes that are triplicated in Ts1Rhr, we observed a significant 101% increase in tumour number in the monosomic mice compared to euploid (Table 1). These results demonstrate that a gene (or combination of genes) in this region is dosage sensitive in both directions with respect to the effect on tumour number

The 33 genes at dosage imbalance in Ts1Rhr and Ms1Rhr mice include several possible candidates for the tumour number effect (Supplementary Table 1), including the *Ets2* 'proto-oncogene'. Although generally considered a 'pro-cancer' gene, *Ets2* has several

Table 1| Average numbers of intestinal tumours in aneuploid and euploid mice at 120 days of age

	Average no. of tumours	s.d.	No. of mice	*t*-test significance (*P* value)
Either *Mom1* allele				
Euploid	45.4	29.9	24	0.008
Ts65Dn	23.8	14.2	10	
$Mom1^{s/s}$				
Euploid	62.6	26.4	14	0.0028
Ts65Dn	31.2	13.7	6	
$Mom1^{s/r}$				
Euploid	21.3	13.3	10	0.105
Ts65Dn	12.8	5.0	4	
Segmental aneuploidies*				
Euploid (B6)	107.3	45.0	16	0.043
Ts1Rhr (B6)	79.6	29.9	21	
Euploid (B6/C3H)	37.0	16.0	9	0.048
Ms1Rhr (B6/C3H)	74.4	39.7	7	

* Genetic background is shown in parentheses. Ts65Dn and euploid controls are B6/C3H (Supplementary Fig. 3).

[1]Department of Physiology and The Institute for Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA. [2]Department of Molecular and Cellular Biochemistry, Ohio State University, Columbus, Ohio 43210, USA. †Present address: Department of Environmental Health Sciences, Bloomberg School of Public Health, Baltimore, Maryland 21205, USA.

activities consistent with a role in repressing the early stages of transformation[10,11]. We performed a three-way cross to produce mice carrying Apc^{Min} that were either euploid or had the Ts1Rhr segmental trisomy, and which segregated an allele of *Ets2* that deletes exons 3–5 and fails to produce functional Ets2 protein (F.L. and M.C.O., manuscript in preparation). Tumours were counted at 120 days (Fig. 1). This independent cohort of mice replicated the observation (Table 1) that trisomy for three copies of *Ets2* and 32 flanking genes in Ts1Rhr results in a significantly reduced tumour incidence, from a mean of 100.8 to 53.9 ($P = 0.001$). However, when *Ets2* was returned to the normal two copy level in mice that were still trisomic for the 32 flanking genes (Apc^{Min}, $Ets2^{+/-}$, Ts1Rhr), average tumour number increased significantly to 81.2 ($P = 0.012$). Thus, a substantial portion though not all of the tumour repression in Ts1Rhr is accounted for by the extra copy of *Ets2*.

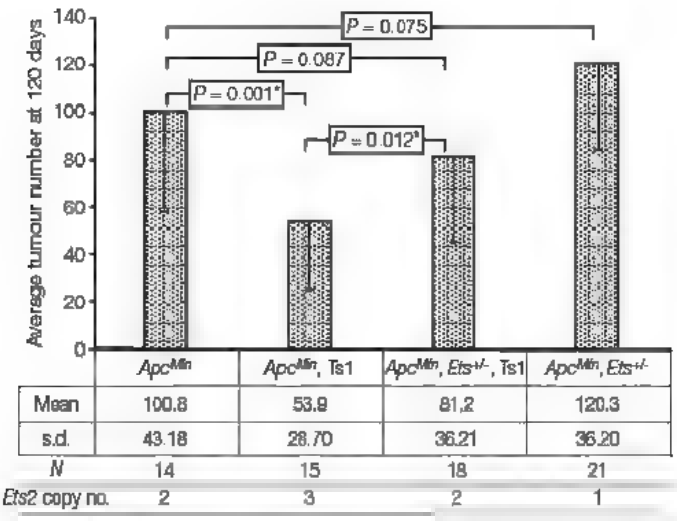
Mice that carried a single copy of *Ets2* in a euploid background showed a substantial, 20% increase in tumour frequency ($P = 0.075$), reminiscent of the increase in tumours in Ms1Rhr mice, which carry a single copy of this gene. These mice developed severe disease much earlier than mice of other genotypes and several did not survive long enough for tumours to be counted. Thus this difference in tumour number is probably under-represented. *Ets2* messenger RNA and protein levels corresponded directly to gene copy number in all of the genotypes (Supplementary Fig. 4).

The size of tumours in a given genetic background provides one indicator of tumour initiation and growth rates. We compared the size of tumours between trisomic and euploid Apc^{Min} mice (Fig. 2a). Ts65Dn, $Mom1^{s/s}$ mice showed a significant 34% reduction in average and median tumour size at 120 days compared to euploid ($P < 0.005$). Note that Ts65Dn mice in this experiment had 48% fewer tumours than did euploid animals, a significantly lower level that replicates in this independent cross the reduction in tumour number reported for the independent cohort of mice represented in Table 1 ($P < 0.04$, $N = 4$ euploid, 5 Ts65Dn).

To determine whether this difference was evident earlier in the course of tumour formation, intestines of trisomic and euploid mice carrying the Apc^{Min} allele were immunostained for β-catenin at 60 days of age (Supplementary Fig. 2)[12]. As at 120 days of age, the number and average size of tumours in Ts65Dn mice was significantly less than in their euploid counterparts (Fig. 2b). No tumours were seen at 30 days of age in two euploid or one trisomic Apc^{Min} mouse after β-catenin staining. Thus the repression of tumour number and size in Ts65Dn mice was evident early in tumour formation.

In contrast to Ts65Dn mice, tumour size was not different from euploid in either Ts1Rhr or Ms1Rhr mice (data not shown). The absence of a tumour size phenotype even though tumour number is reduced in Ts1Rhr mice indicates that multiple genes on Mmu16 (and Hsa21) may contribute to different aspects of tumour repression caused by trisomy.

For 50 yr, epidemiological studies examining rates of solid tumours in individuals with Down's syndrome have reached discrepant conclusions about whether trisomy is protective against cancer[2,13–16] (Supplementary Table 2). Although our demonstration of tumour repression owing to gene dosage applies specifically to the role of trisomy and especially *Ets2* dosage in Apc-induced tumours, it provides biological evidence supporting the protective effect of trisomy. It will be important to determine the range of cancer types



	Apc^{Min}	Apc^{Min}, Ts1	Apc^{Min}, $Ets^{+/-}$, Ts1	Apc^{Min}, $Ets^{+/-}$
Mean	100.8	53.9	81.2	120.3
s.d.	43.18	28.70	36.21	36.20
N	14	15	18	21
Ets2 copy no.	2	3	2	1

Figure 1 | *Ets2* dosage is substantially responsible for tumour number repression or increase. Average tumour number at 120 days is measured for the four genotypes, error bars indicate s.d. Number of mice analysed, *P* value and the gene copy number of *Ets2* in each strain are indicated. *, statistical significance by Student's *t*-test of the designated pair. Although the increased tumour number in euploid $Ets2^{+/-}$ mice at 120 days did not reach a formal level of statistical significance, this result underestimates the impact of reduced *Ets2* dosage, because four Apc^{Min}, $Ets2^{+/-}$ mice became sick and were euthanized before tumours could be counted at 120 days. None of the 47 mice representing the other 3 genotypes died before 120 days.

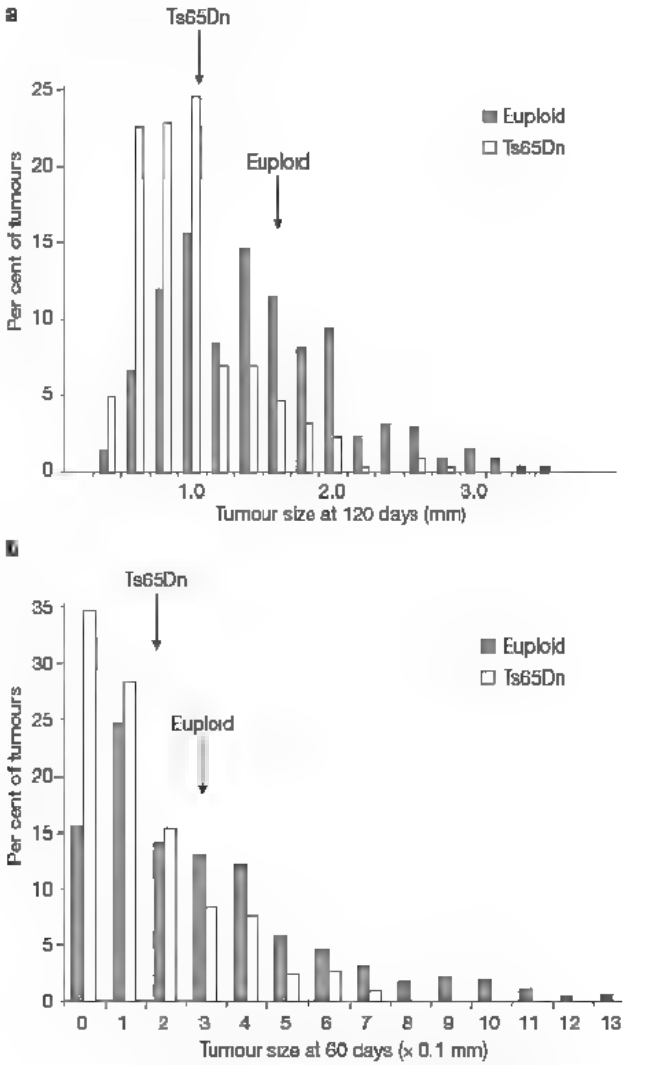


Figure 2 | Tumour growth and number are reduced in Ts65Dn mice. Distribution of tumour sizes for trisomic (open bars) and euploid (closed bars) mice. **a**, At 120 days of age, tumour number is reduced and tumours are significantly smaller in Ts65Dn $Mom1^{s/s}$ than in euploid mice. Mean tumour size is reduced by 34%; Ts65Dn = 0.91 mm, euploid = 1.38 mm ($P = 0.005$, $N = 347$ and 577 tumours for Ts65Dn and euploid, respectively). **b**, At 60 days, the number of tumours identified after staining with β-catenin is significantly reduced in Ts65Dn ($P = 0.037$) and mean tumour size is reduced 36%; mean = 18 μm in trisomic and 28 μm in euploid mice ($P = 0.029$, $n = 346$ and 636 tumours for Ts65Dn and euploid, respectively). Arrows indicate mean tumour size in each genotype

and the range of dosage-sensitive genes that contribute to this protective effect in different tissues.

Notable among the Hsa21 genes that have been implicated in pro- or anti-tumorigenesis is endostatin, an inhibitor of angiogenesis that has been shown to be a potent inhibitor of tumour growth in model systems[17]. Elevated expression of another Hsa21 gene, *RCAN1*, can reduce endothelial cell proliferation and angiogenesis, affecting size and vascularity of xenografted tumours[18]. However, *Rcan1* is not trisomic in Ts1Rhr, and the *Col18a1* gene (which encodes endostatin) is not triplicated in either Ts65Dn or Ts1Rhr. Therefore, these genes do not account for the reduction in tumour number seen here.

Two general implications that stem from the observation that trisomy and specifically *Ets2* dosage can repress or promote tumour growth are worth special note. First, repression of tumorigenesis when *Ets2* expression is elevated may in fact be a characteristic of many genes identified previously as oncogenes or tumour suppressor genes. Natural variation in average expression levels of ETS family (or other) repressor genes may exist in tumour-prone families without a known molecular basis for a high cancer frequency (reduced expression of *Ets2*) or in cancer-resistant families (elevated expression). This phenomenon might be exploited to identify a pharmacological-based approach to tumour protection.

Second, previous observations about the role of the *ETS2* proto-oncogene in cancer could not have predicted that elevation of expression beyond euploid levels would provide a natural repression of tumour formation and growth. If trisomy for Hsa21 was not viable, the correlation of increased gene expression with lower solid tumour frequency would not occur in a systematic manner and may not have been observed for some time. The implication for promoting tumour resistance in all people on the basis of gene dosage of 'oncogenes' is thus a product of the genetic heritage of those with Down's syndrome.

METHODS SUMMARY

C57BL/6J-*Apc*^{Min} mice (herein *Apc*^{Min}) and B6EiC3Sn *a/A*-Ts(17^{16})65Dn (herein Ts65Dn) mice were purchased from the Jackson Laboratory and genotyped as described[19]. B6.Dup(Cbr1-ORF9)1Rhr mice (herein Ts1Rhr)[9] were backcrossed eight or more generations onto C57Bl/6J (B6). B6C.3Del(16Cbr1-ORF9)1Rhr (herein Ms1Rhr) and Ts65Dn mice were maintained as an advanced intercross between B6 and C3H. For tumour analysis, mice were euthanized at 120 ± 2 days, intestines were placed in fresh PBS, and tumours counted under 20× magnification. Tumour size was determined for the longest axis, using an eyepiece reticule. Statistical significance was determined using a Student's *t*-test. Detailed methods are in Supplementary Information and Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 31 August; accepted 31 October 2007.

1. Satgé, D. *et al.* A tumor profile in Down syndrome. *Am. J. Med. Genet.* 78, 207–216 (1998).
2. Yang, Q., Rasmussen, S. A. & Friedman, J. M. Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. *Lancet* 359, 1019–1025 (2002).
3. Reeves, R. H. *et al.* A mouse model for Down Syndrome exhibits learning and behaviour deficits. *Nature Genet.* 11, 177–183 (1995).
4. Gardiner, K., Fortna, A., Bechtel, L. & Davisson, M. T. Mouse models of Down syndrome: how useful can they be? Comparison of the gene content of human chromosome 21 with orthologous mouse genomic regions. *Gene* 318, 137–147 (2003).
5. Su, L. K. *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 256, 668–670 (1992).
6. Yamada, Y. & Mori, H. Multistep carcinogenesis of the colon in *Apc*$^{Min/+}$) mouse. *Cancer Sci.* 98, 6–10 (2007).
7. Dietrich, W. F. *et al.* Genetic identification of *Mom-1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. *Cell* 75, 631–639 (1993).
8. MacPhee, M. *et al.* The secretory phospholipase A2 gene is a candidate for the *Mom1* locus, a major modifier of *Apc*^{Min}-induced intestinal neoplasia. *Cell* 81, 957–966 (1995).
9. Olson, L. E., Richtsmeier, J. T., Leszl, J. & Reeves, R. H. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science* 306, 687–690 (2004).
10. Hsu, T., Trojanowska, M. & Watson, D. K. Ets proteins in biological control and cancer. *J. Cell. Biochem.* 91, 896–903 (2004).
11. Wolvetang, E. J. *et al.* Overexpression of the chromosome 21 transcription factor Ets2 induces neuronal apoptosis. *Neurobiol. Dis.* 14, 349–356 (2003).
12. Roberts, R. B. *et al.* Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. *Proc. Natl Acad. Sci. USA* 99, 1521–1526 (2002).
13. Day, S. M., Strauss, D. J., Shavelle, R. M. & Reynolds, R. J. Mortality and causes of death in persons with Down syndrome in California. *Dev. Med. Child Neurol.* 47, 171–176 (2005).
14. Hill, D. A. *et al.* Mortality and cancer incidence among individuals with Down syndrome. *Arch. Intern. Med.* 163, 705–711 (2003).
15. Patja, K., Pukkala, E., Sund, R., Iivanainen, M. & Kaski, M. Cancer incidence of persons with Down syndrome in Finland: a population-based study. *Int. J. Cancer* 118, 1769–1772 (2006).
16. Goldacre, M. J., Wotton, C. J., Seagroatt, V. & Yeates, D. Cancers and immune related diseases associated with Down's syndrome: a record linkage study. *Arch. Dis. Child.* 89, 1014–1017 (2004).
17. O'Reilly, M. S. *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88, 277–285 (1997).
18. Minami, T. *et al.* Vascular endothelial growth factor- and thrombin-induced termination factor, Down syndrome critical region-1, attenuates endothelial cell proliferation and angiogenesis. *J. Biol. Chem.* 279, 50537–50554 (2004).
19. Moore, C. S. *et al.* Integration of cytogenetic with recombinational and physical maps of mouse chromosome 16. *Genomics* 59, 1–5 (1999).
20. The Jackson Laboratory Genotyping protocol for *Apc* (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype=protocol&protocol_id=529#133).
21. Smith, J. L. *et al.* ets-2 is a target for an akt (Protein kinase B)/jun N-terminal kinase signaling pathway in macrophages of *motheaten-viable* mutant mice. *Mol. Cell. Biol.* 20, 8026–8034 (2000).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements The authors thank L. Siracusa for advice regarding the *Apc*^{Min} system, C. Dang for important discussions regarding cancer models, and R. Roper for statistical advice. This work was supported by National Institute of Child Health and Development and National Cancer Institute awards (M.C.O. and R.H.R.).

Author Contributions T.E.S. and R.H.R. designed the experiments, T.E.S. and A.Y. managed husbandry and collected tumour data, which were analysed by T.E.S., A.Y. and R.H.R.; F.L. and M.C.O. designed the *Ets2* conditional knockout mice; and A.Y., F.L. and M.C.O. analysed *Ets2* expression. R.H.R. wrote the paper with substantial input from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.H.R. (rreeves@jhmi.edu).

LETTERS

NUMB controls p53 tumour suppressor activity

Ivan N. Colaluca[1,2], Daniela Tosoni[1,2], Paolo Nuciforo[1], Francesca Senic-Matuglia[1], Viviana Galimberti[2], Giuseppe Viale[2,3], Salvatore Pece[1,2,3] & Pier Paolo Di Fiore[1,2,3]

NUMB is a cell fate determinant, which, by asymmetrically partitioning at mitosis, controls cell fate choices by antagonising the activity of the plasma membrane receptor of the NOTCH family[1]. NUMB is also an endocytic protein[2], and the NOTCH–NUMB counteraction has been linked to this function[3,4]. There might be, however, additional functions of NUMB, as witnessed by its proposed role as a tumour suppressor in breast cancer[5]. Here we describe a previously unknown function for human NUMB as a regulator of tumour protein p53 (also known as TP53). NUMB enters in a tricomplex with p53 and the E3 ubiquitin ligase HDM2 (also known as MDM2), thereby preventing ubiquitination and degradation of p53. This results in increased p53 protein levels and activity, and in regulation of p53-dependent phenotypes. In breast cancers there is frequent loss of NUMB expression[5]. We show that, in primary breast tumour cells, this event causes decreased p53 levels and increased chemoresistance. In breast cancers, loss of NUMB expression causes increased activity of the receptor NOTCH[5]. Thus, in these cancers, a single event—loss of NUMB expression—determines activation of an oncogene (NOTCH) and attenuation of the p53 tumour suppressor pathway. Biologically, this results in an aggressive tumour phenotype, as witnessed by findings that NUMB-defective breast tumours display poor prognosis. Our results uncover a previously unknown tumour suppressor circuitry.

p53 is one of the major tumour suppressor proteins[6]. Mutations in the *p53* gene are detected in ~50% of human cancers[7]. Indirect mechanisms also lead to p53 inactivation. HDM2 binds to p53 and hinders its transcriptional activity[8]. In addition, HDM2 regulates p53 half-life through its E3 ubiquitin-ligase activity[9,10]. The tumour suppressor ARF (alternative reading frame, and encoded by the *INK4a/ARF* locus; *INK4a* is also called *CDKN2A*) binds to HDM2 and interferes with its activity, thereby stabilizing p53 (ref. 11). Thus, in human tumours, amplification of the *HDM2* gene[12] or loss of the ARF protein[13] result in defective p53 activity. In breast tumours, p53 mutations and amplification of *HDM2* are not as frequent as in other tumours, being detected in ~20% and <10% of cases, respectively[14–16]. Similarly, homozygous deletions or mutations of the *INK4a/ARF* locus are rare[17–19]. Although epigenetic mechanisms might contribute to altered expression of HDM2 or ARF[14], other unknown circuitries of regulation of p53 might be subverted in breast tumours. NUMB is a candidate for this role, in that it binds to HDM2 (ref. 20) and is frequently underexpressed in breast cancers[5]

Previous work has shown interaction *in vivo* between overexpressed NUMB and HDM2 (ref. 20). Also the two endogenous proteins can be co-immunoprecipitated from cellular lysates of normal mammary MCF10A cells (Fig. 1a). In addition, the four described isoforms of NUMB all interact with HDM2 (Supplementary Fig. 1a). NUMB also co-immunoprecipitates with p53 (Fig. 1a). This is compatible with the existence *in vivo* of multiple binary complexes (NUMB–HDM2, NUMB–p53 and HDM2–p53), or of a tricomplex NUMB–HDM2–p53. In *in vitro* binding assays with purified proteins, all three binary complexes could form, indicating direct interactions between the three proteins (Supplementary Fig. 1b). The presence of nutlin, which inhibits the HDM2–p53 interaction[21], prevented the formation of the HDM2–p53 complex, but not of the HDM2–NUMB or p53–NUMB complexes (Fig. 1b). Thus, the NUMB–p53 and NUMB–HDM2 surfaces of interaction are distinct, at least in part, from that of the HDM2–p53 interaction. This pointed to the possibility of formation of a tricomplex *in vivo* (which might coexist with the various binary complexes)—a possibility confirmed by sequential immunoprecipitation experiments with endogenous (Fig. 1c) or overexpressed (Supplementary Fig. 1c) proteins. Nutlin and cisplatin strongly decreased the NUMB–HDM2, but not the NUMB–p53, interaction (Supplementary Fig. 1d, e) These results argue that the stability of the tricomplex is affected by agents disrupting the HDM2–p53 interaction and that it is regulated in response to stress signals.

We analysed the effects of *NUMB* knockdown (*NUMB*-KD) on p53 by targeting two different sequences in *NUMB* using short interfering RNA (siRNA) or short hairpin RNA (shRNA), respectively. Both methods yielded 80–90% decrease in NUMB levels and an approximately twofold decrease in the p53 steady-state levels (Fig. 1d and Supplementary Fig. 2a). This effect was NUMB-specific because ablation of the related protein NUMBL (NUMB-like) did not affect p53 levels (Fig. 1d) Importantly, approximately threefold-higher doses of genotoxic drugs (Fig. 1e, f and Supplementary Fig. 2b) were needed to induce, in knockdown cells, levels of p53 comparable to those induced in wild-type cells. Similar results were obtained in primary normal human mammary cells (Supplementary Fig. 2c). The reduced levels of p53 on cisplatin treatment were paralleled by a reduction in the levels of Ser 15-phosphorylated p53 (Fig. 1f), a marker of the activation status of p53. Furthermore, we observed marked reductions in the expression of several p53 transcriptional targets (Fig. 1f, g). Thus, in *NUMB*-KD cells, the overall activation status of p53 after DNA damage is reduced.

In *NUMB*-KD cells, *p53* messenger RNA levels were not altered (Fig. 2a), arguing for NUMB-mediated regulation of p53 at the post-transcriptional level. Indeed, the half-life of p53 was reduced in *NUMB*-KD versus control cells, from ~60 to ~20 min (Fig. 2b, c). The half-life of HDM2 was not affected, arguing that *NUMB*-KD does not primarily affect HDM2 stability (Fig. 2b, c).

The simultaneous silencing of *NUMB* and *HDM2* restored p53 to levels indistinguishable from that of control cells or *HDM2*-KD cells (Fig. 2d). Nutlin stabilizes and activates p53 (ref. 21). Nutlin treatment of MCF10A cells resulted in increased steady-state levels of p53 and of HDM2 (Fig. 2e), which confirms that the accumulated p53 protein is transcriptionally active. More importantly, nutlin reversed the effects of *NUMB*-silencing on p53 levels (Fig. 2e). Thus, there is a requirement for HDM2 in the regulation of p53 by NUMB

[1] FOM, the FIRC Institute for Molecular Oncology Foundation, Via Adamello 16, 20139 Milan, Italy [2] European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy
[3] Dipartimento di Medicina, Chirurgia ed Odontoiatria, Università degli Studi di Milano, 20122 Milan, Italy

HDM2 regulates p53 turnover through its E3 activity. In *NUMB*-KD cells, we detected enhanced ubiquitination of p53, which was inhibited by nutlin (Fig. 2f), arguing for a direct counteraction of NUMB over the p53-ubiquitinating activity of HDM2. This was confirmed in *in vitro* assays in which glutathione *S*-transferase (GST)–p53 was used as a substrate for the E3 activity of HDM2. Under these conditions, ubiquitinated p53 was readily detectable (Fig. 2g). However, this effect was abolished in the presence of purified NUMB or nutlin (Fig. 2g; see Supplementary Fig. 3 for possible models for action of NUMB).

NUMB inhibits NOTCH activity. Thus, it was important to prove that the observed effects were not a consequence of deregulated NOTCH activity. We treated *NUMB*-KD cells with inhibitors of presenilin/γ-secretase (known as γ-secretase inhibitors, GSI) to abolish NOTCH activity. GSI had no significant effect on p53 levels in *NUMB*-KD or control cells (Fig. 2h and Supplementary Fig. 4a), ruling out participation of NOTCH to the observed NUMB regulation of p53. This was confirmed using gain-of-function mutants of NOTCH (Supplementary Fig. 4b).

We tested whether the NUMB–HDM2 counteraction was relevant to p53-dependent transcriptional activity. HDM2 significantly inhibited the trans-activating ability of p53 on a luciferase reporter gene. However, NUMB restored, albeit not completely, this ability in a dose-dependent fashion (Fig. 2i).

Perturbation of NUMB levels should result in alterations in p53-mediated responses to DNA damage. To monitor DNA damage, we analysed the levels of phosphorylation at serine 139 of histone H2AFX (γ-H2AX) in cells treated with cisplatin, because prolonged persistence of γ-H2AX is considered to be a marker of persistent DNA damage[22,23]. MCF10A cells were treated and then washed free of the drug and monitored for up to 48 h. In both *p53*-KD (Fig. 3a) and *NUMB*-KD (Fig. 3b, c) cells, we detected higher levels of γ-H2AX than in control cells, and demonstrated persistence of γ-H2AX during the cisplatin chase.

We also tested the effects of *NUMB* or *p53* ablation on perturbations of the cell cycle induced by genotoxic drugs. In MCF10A cells, cisplatin induced an S-phase block; this was, however, independent of p53 or NUMB, and thus not informative for our purposes (Fig. 3d and Supplementary Fig. 5). However, doxorubicin and SN38 induced a G2/M block and an S-phase block, respectively, which were partially rescued by *NUMB*-KD or *p53*-KD (Fig. 3d). This effect is better illustrated by correcting for the initial percentage of cells in the various phases of the cycle (Fig. 3d, right-most panel), because the ablation of NUMB or p53 caused some alterations in the cell cycle already at steady state.

After treatment and washout with doxorubicin or SN38, MCF10A cells did not efficiently re-enter the cell cycle for at least 20 h (Fig. 3e), probably owing to checkpoint activation. Conversely, *NUMB*-KD cells and *p53*-KD cells displayed accelerated exit from the blocked phase (Fig. 3e). Moreover, doxorubicin and SN38 caused a marked block of cell proliferation, which was partly alleviated by the silencing of *NUMB* or *p53*. In addition, even after washout of the drugs, the proliferation rate was more sustained in *NUMB*-KD and *p53*-KD cells compared to control MCF10A cells (Fig. 3f).

We then performed experiments under conditions of NUMB overexpression. Expression of NUMB–GFP (green fluorescent protein) in MCF10A cells increased the levels of p53 in both unstressed cells and cisplatin- or doxorubicin-treated cells (Supplementary Fig. 6a, b). The moderate, albeit reproducible, increase in p53 possibly reflects the fact that proliferating cells can tolerate only limited amounts of

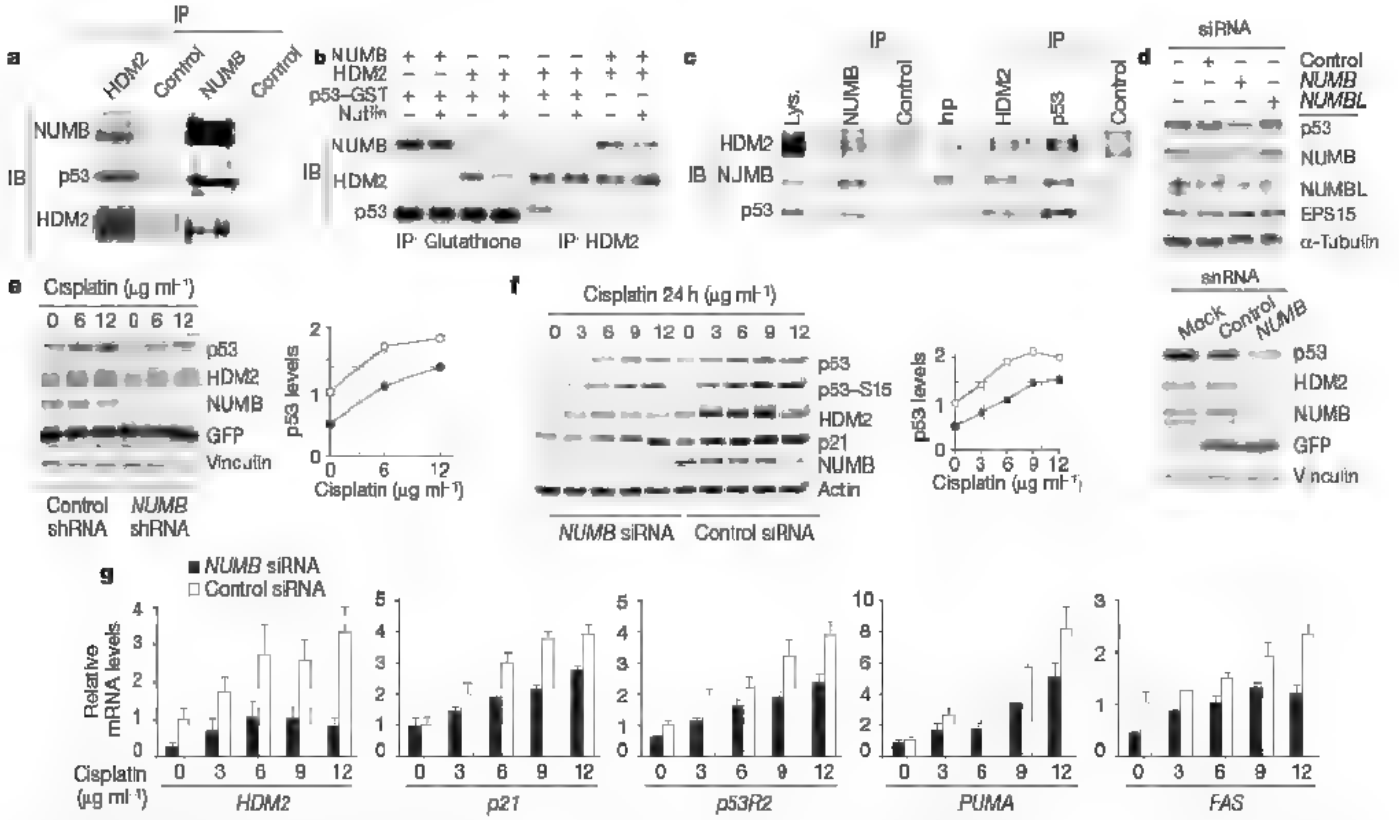


Figure 1 | NUMB interacts with and regulates p53. **a**, MCF10A lysates (3 mg) were immunoprecipitated (IP) and immunoblotted (IB). The control was an irrelevant antibody. **b**, Pure HDM2, GST–p53 and NUMB were mixed (3.2 nM each) and the solution was immunoprecipitated and immunoblotted as shown. **c**, Left, lysates (40 mg) from U2OS cells were immunoprecipitated with anti-NUMB (control, irrelevant antibody) and an aliquot (one-twenty-fifth) of the immunoprecipitate and immunoblot as shown. Right, the immunoprecipitate was eluted with the immunogenic NUMB peptide (amino acids 537–551), the immunoprecipitate and immunoblot were as indicated. Inp, one-fortieth of the eluate; Lys., lysate. **d**, MCF10A lysates, transfected as shown, were immunoblotted as indicated. **e**, **f**, MCF10A cells, transfected as shown, were exposed to cisplatin (24 h). The immunoblot was as indicated. Right, quantification (mean of three experiments) of p53 induction in control (open circles) and *NUMB*-KD cells (filled circles). **g**, Quantitative PCR with reverse transcription (RT–PCR) in control siRNA (open bars) and *NUMB*-KD (filled bars) MCF10A cells. Values represent mean (control siRNA/no cisplatin = 1) ± s.d. from two experiments. Cisplatin, 8 h.

p53. NUMB overexpression also enhanced p53-dependent transcriptional activity, and prolonged p53 half-life (from ~60 to ~120 min) (Supplementary Fig. 6a–d).

The above results demonstrate that NUMB overexpression increases p53 stability and activity, predicting enhancement of p53-mediated responses to genotoxicity, such as apoptosis. Thus, we monitored activation of caspases[24]. NUMB–GFP-transfected MCF10A cells displayed an approximately threefold-higher level of activated caspase-3 compared to control cells in response to cisplatin-induced DNA damage—an effect that was abolished by silencing of *p53* (Supplementary Fig. 6e, f). These results show that NUMB levels are relevant to the p53-mediated cellular responses.

In breast tumours, loss of NUMB expression is frequently detected[5]. These tumours should harbour reduced p53 levels and impaired p53-mediated responses. We addressed these issues in primary human breast tumour cells. These cells[5] can be cultivated from tumours displaying low or absent levels of NUMB (class 1 tumour cells) or normal levels of NUMB (class 3). We selected eight primary cultures (four of each for class 1 and class 3). The p53 coding sequence was normal in all selected primary cells (not shown).

In class 1 compared with class 3 cells, the steady-state levels of p53 were reduced (Fig. 4a) owing to increased proteasomal degradation, as shown by comparable *p53* mRNA levels in class 1 and 3 (Fig. 4b), and to restoration of p53 in class 1 cells by the proteasome inhibitor MG132 (Fig. 4a). The reduction in p53 levels was caused by loss of NUMB, by means of HDM2. Indeed, forced re-expression of NUMB–GFP (Fig. 4c) or silencing of *HDM2* (Fig. 4d) restored normal p53 levels in class 1, whereas it had a limited effect, as expected, in class 3 cells.

Deficient p53 activity is associated with resistance to the cytotoxic effects of chemotherapy[25]. Thus, NUMB-defective breast tumours should show resistance to genotoxic anticancer drugs. Indeed, class 1 cells exhibited higher resistance to cisplatin than class 3 cells (Fig. 4e). Re-expression of NUMB in class 1 cells restored responsiveness to cisplatin to levels comparable to those of control class 3 cells (Fig. 4e). NUMB-silencing in class 3 cells increased resistance to the drug to levels comparable to those of control class 1 cells (Fig. 4e). Nutlin restored the susceptibility of class 1 cells to cisplatin (Fig. 4e), and reverted the effects of NUMB ablation in class 3 cells (Fig. 4e), again implicating the HDM2–p53 circuitry.

Finally, we analysed a cohort of 443 breast cancer patients who received adjuvant chemotherapy. We found that NUMB status was inversely correlated with the major clinical and pathological parameters indicative of biologically aggressive neoplastic disease (Supplementary Table 1), and that it behaved as an independent predictor of poor prognosis (Fig. 4f). In conclusion, in breast tumours there is frequent loss of NUMB expression[5], and this event causes decreased p53 activity. Moreover, loss of NUMB expression is associated with poor prognosis, further arguing its clinical relevance. We note that lack of NUMB also leads to increased NOTCH activity[5]. Thus, the alteration of NUMB concomitantly leads to the activation of an oncogene, NOTCH, and to the attenuation of a tumour suppressor, p53.

Many questions await answers. It remains to be established where NUMB, HDM2 and p53 interact. This is not trivial because HDM2 and p53 are by-and-large nuclear proteins whereas NUMB is in the cytoplasm, mostly associated to biomembranes[2]. Similarly, our findings ask whether endocytosis participates to the regulation of p53, because NUMB is an endocytic protein. Of note, it has been shown

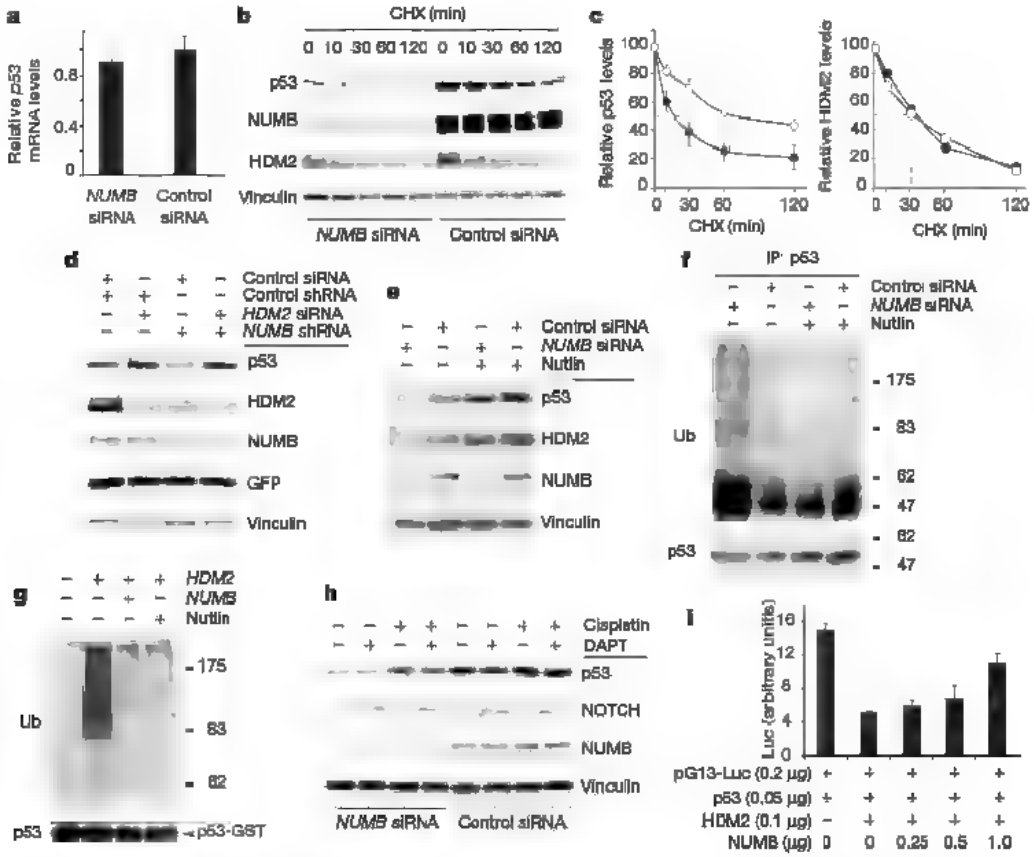


Figure 2 | NUMB regulates HDM2-mediated degradation of p53. **a**, *p53* mRNA levels in control-siRNA and *NUMB*-siRNA MCF10A cells. Values represent the mean ± s.d. (control siRNA = 1) from two experiments. **b**, **c**, MCF10A cells, transfected as shown, were treated with cycloheximide (CHX). Immunoblot was as indicated. In **c**, quantification of p53 and HDM2 levels in control-siRNA (open circles) and *NUMB*-siRNA (filled circles) cells are shown; values are expressed relative to time 0 (normalized to vinculin), and represent, in the case of p53, the mean ± s.d. of three experiments. **d**, **e**, **f**, Lysates from MCF10A cells, transfected and treated as indicated, were immunoprecipitated and immunoblotted as shown. In **f**, p53 levels were normalized by loading proportionally different amounts of cell extracts. **g**, GST–p53 was subjected to *in vitro* ubiquitination assay as indicated. Detection was in the immunoblot (Ub, anti-ubiquitin antibody). **h**, Lysates from MCF10A cells, transfected and treated as shown, were immunoblotted as indicated. **i**, Luciferase assay in U2OS cells transfected as indicated. Results represent mean ± s.d. from three experiments.

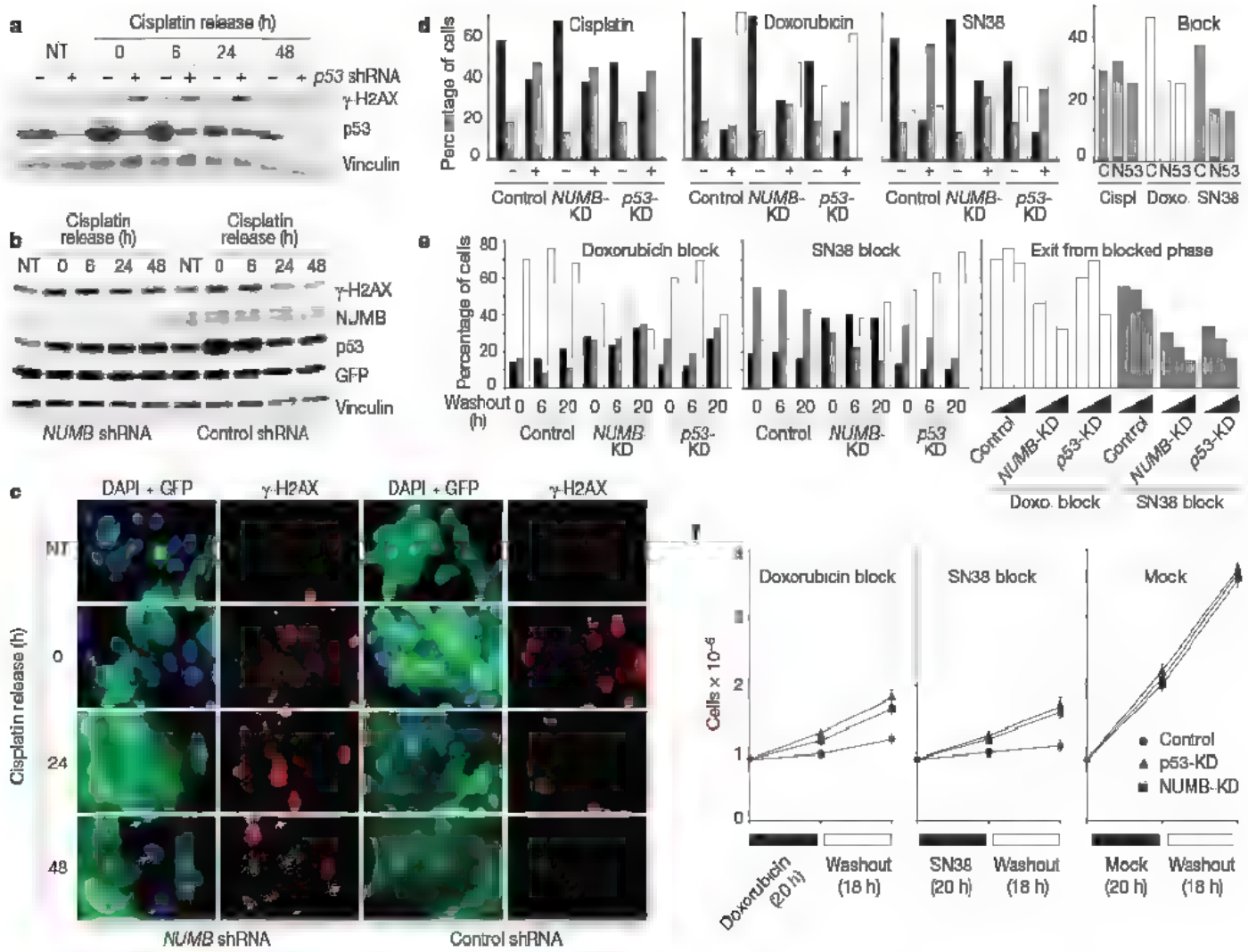


Figure 3 | *NUMB* silencing alters the p53-mediated response to DNA damage. **a**, **b**, MCF10A cells, in which *p53* (*p53* shRNA, **a**) or *NUMB* (*NUMB* shRNA, **b**) had been silenced, were treated with cisplatin (6 μg ml^{-1} for 15 h) and released in cisplatin-free medium. Immunoblot was as indicated. NT, not treated. **c**, γ-H2AX (red) in MCF10A cells, infected and treated as in **b**. Green (GFP), transduced cells. Asterisks, GFP-positive cells with persistent γ-H2AX staining. Arrowheads, non-infected cells with loss of γ-H2AX staining. Blue, 4,6-diamidino-2-phenylindole (DAPI). Original magnification, ×40. **d**, MCF10A, silenced with *p53* shRNA (*p53*-KD), *NUMB* shRNA (*NUMB*-KD) or control shRNA (Control) were mock-treated ( ) or treated (+) for 24 h (cisplatin, 9 μg ml^{-1}; doxorubicin, 0.05 μg ml^{-1}; SN38, 10 ng ml^{-1}). Bars: solid, G1; grey, S; empty, G2/M. In the far-right 'Block' graph, values of the blocked phases are reported (cisplatin and SN38, S; doxorubicin, G2/M) in *NUMB*-KD (N), *p53*-KD (53) or control (C) cells. Values are expressed after subtracting cells in the same phase under non-treated conditions. **e**, MCF10A cells, silenced and treated as in **d**, were released in drug-free medium (washout) for 6 or 20 h, followed by fluorescence-activated cell sorting. For the 'Exit from the blocked phase' graph, values of the blocked phases (doxorubicin, G2/M; SN38, S) are reported versus time of drug washout (0, 6 or 20 h, triangles). **f**, MCF10A cells, silenced as in **d**, were treated for 20 h and then released in drug-free medium for 18 h. Cells were counted at the indicated times. **d**–**f** are representative of at least three experiments in triplicates.

that other endocytic proteins control various aspects of p53-mediated functions: dynamin 2 induces p53-dependent apoptosis[26], and the clathrin heavy chain promotes p53-mediated transcription[27] (see also Supplementary Discussion).

Finally, because NUMB is involved in binary fate decisions[1], a relevant question is whether NUMB, HDM2 or p53 are involved in the homeostasis of mammary stem cells, and in its subversion in tumours. The role of p53 in stem cells has been widely investigated, mostly in light of the induction of cellular senescence by p53, which in turn can be linked to the depletion of stem cells and to organism ageing[28]. Our data raise the possibility that p53, because of the NUMB liaison, is involved in the initial process that sits at the heart of stem cell fate (that is, asymmetric cell division). Indeed, a role for p53 as a cell-autonomous asymmetric kinetics control gene has been proposed[29], which might be due to its involvement in regulating the linked phenomenon of immortal DNA strand cosegregation[30]. Thus, our data put forward the possibility that an additional mechanism of tumorigenesis, caused by the lack of the p53/NUMB axis, is the skewing of stem cell division towards a symmetric pattern.

METHODS SUMMARY

Cultivation of primary epithelial cells was performed as described previously[5]. Procedures for immunofluorescence, immunoblotting and immunoprecipitation were also performed as described previously[5]. A list of the antibodies and reagents used is in Supplementary Information.

pG13-Luc and expression vectors for *p53* and *HDM2* were a gift of K. Helin. *p53* shRNA pSUPER was extracted from a shRNA library (a gift from R. Bernard). The retroviral PINCO–NUMB–GFP vector was as described[5]. The lentiviral pLL3.7 vector was a gift from L. Van Parijs. All constructs used in this study were sequence-verified. Procedures for retroviral infection and luciferase assays were also described[5]. Procedures for lentiviral infection are shown at http://web.mit.edu/ccr/labs/jacks/protocols/pll37.htm.

Specific siRNA for *NUMB* and controls were as described previously[5]. *NUMBL*- and *HDM2*-specific siRNA were from Dharmacon. *HDM2*, 5′-GCCACAAAUCUGAUAGUAU-3′; *NUMBL*, 5′-ACGCCUUCUGCUCAGCCGC-3′.

Real-time PCR for *p53*, *HDM2*, *p21* (also known as *CDKN1A*), *p53R2* (also known as *RRM2B*), *PUMA* (also known as *BBC3*) and *FAS* was performed using the TaqMan Gene Expression Assays Identification, Hs00153349-m1, Hs00242813-m1, Hs00355782-m1, Hs00153085_m1, Hs00248075_m1 and Hs00163653_m1, respectively (Applied Biosystems).

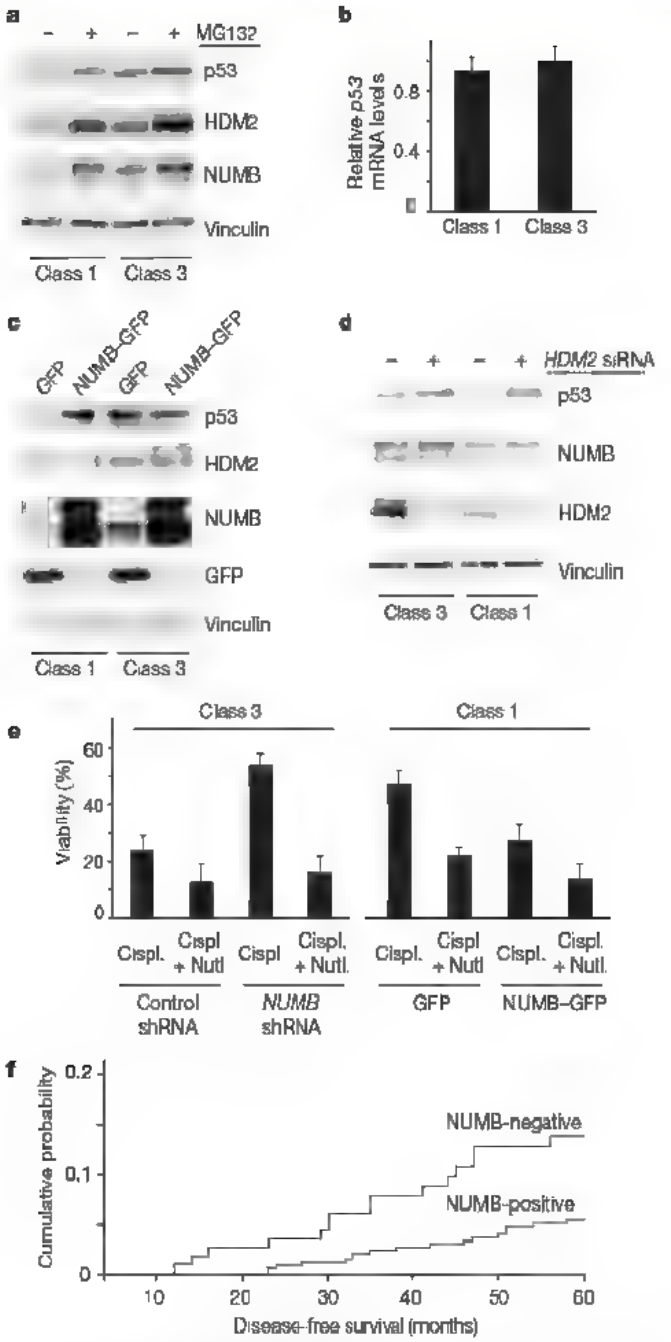


Figure 4 | Loss of NUMB in human breast tumours determines decreased p53, enhanced chemoresistance and predicts poor prognosis. a, Class 1 and class 3 cells were treated with MG132 (+). Immunoblot was as indicated. **b**, *p53* transcripts. Results represent mean (normalized to class 3) ± s.d. from four tumours. **c**, Class 1 and class 3 cells were transduced as shown. Immunoblot was as indicated. **d**, Class 1 and class 3 cells were transfected with *HDM2* siRNA (+) or control siRNA (–). Immunoblot was as indicated. **e**, Class 1 and class 3 cells were transduced as indicated, treated with cisplatin and nutlin, and analysed for cell viability. Results represent mean ± s.d. from triplicate points. In **a**, **c**, **d** and **e**, results are representative of four class 1 and four class 3 cultures, from different patients. **f**, NUMB status (as evaluated by immunohistochemistry) and prognosis (as evaluated by cumulative probability of any secondary event) in patients with breast cancer. Kaplan–Meier curves were compared by the Log-rank test. Hazard ratio before adjustment for clinical and pathological features (Cox proportional hazard method), 0.610 (P, 0.0068), hazard ratio after adjustment, 0.651 (P, 0.0291); Log-rank, 0.00387.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 1 March; accepted 23 October 2007.

1. Roegiers, F. & Jan, Y. N. Asymmetric cell division. *Curr. Opin. Cell Biol.* 16, 195–205 (2004).
2. Santolini, E. *et al.* Numb is an endocytic protein. *J. Cell Biol.* 151, 1345–1352 (2000).
3. Berdnik, D., Torok, T., Gonzalez-Gaitan, M. & Knoblich, J. A. The endocytic protein α-adaptin is required for numb-mediated asymmetric cell division in *Drosophila*. *Dev. Cell* 3, 221–231 (2002).
4. Hutterer, A. & Knoblich, J. A. Numb and α-adaptin regulate Sanpodo endocytosis to specify cell fate in *Drosophila* external sensory organs. *EMBO Rep.* 6, 836–842 (2005).
5. Pece, S. *et al.* Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J. Cell Biol.* 167, 215–221 (2004).
6. Vousden, K. H. & Prives, C. p53 and prognosis: new insights and further complexity. *Cell* 120, 7–10 (2005).
7. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. p53 mutations in human cancers. *Science* 253, 49–53 (1991).
8. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 69, 1237–1245 (1992).
9. Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* 387, 296–299 (1997).
10. Kubbutat, M. H., Jones, S. N. & Vousden, K. H. Regulation of p53 stability by Mdm2. *Nature* 387, 299–303 (1997).
11. Zhang, Y., Xiong, Y. & Yarbrough, W. G. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* 92, 725–734 (1998).
12. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L. & Vogelstein, B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 358, 80–83 (1992).
13. Sherr, C. J. Tumor surveillance via the ARF–p53 pathway. *Genes Dev.* 12, 2984–2991 (1998).
14. McCann, A. H. *et al.* Amplification of the *MDM2* gene in human breast cancer and its association with MDM2 and p53 protein status. *Br. J. Cancer* 71, 981–985 (1995).
15. Pharoah, P. D., Day, N. E. & Caldas, C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br. J. Cancer* 80, 1968–1973 (1999).
16. Vestey, S. B. *et al.* $p14^{ARF}$ expression in invasive breast cancers and ductal carcinoma *in situ*—relationships to p53 and Hdm2. *Breast Cancer Res.* 6, R571–R585 (2004).
17. Sharpless, N. E. & DePinho, R. A. The INK4A/ARF locus and its two gene products. *Curr. Opin. Genet. Dev.* 9, 22–30 (1999).
18. Silva, J. *et al.* Analysis of genetic and epigenetic processes that influence $p14^{ARF}$ expression in breast cancer. *Oncogene* 20, 4586–4590 (2001).
19. Silva, J. *et al.* Concomitant expression of *p16INK4a* and *p14ARF* in primary breast cancer and analysis of inactivation mechanisms. *J. Pathol.* 199, 289–297 (2003).
20. Juven-Gershon, T. *et al.* The Mdm2 oncoprotein interacts with the cell fate regulator Numb. *Mol. Cell. Biol.* 18, 3974–3982 (1998).
21. Vassilev, L. T. *et al.* *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303, 844–848 (2004).
22. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* 273, 5858–5868 (1998).
23. Paull, T. T. *et al.* A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol.* 10, 886–895 (2000).
24. Reis, T. & Edgar, B. A. Negative regulation of dE2F1 by cyclin-dependent kinases controls cell cycle timing. *Cell* 117, 253–264 (2004).
25. Lowe, S. W., Ruley, H. E., Jacks, T. & Housman, D. E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 74, 957–967 (1993).
26. Fish, K. N., Schmid, S. L. & Damke, H. Evidence that dynamin-2 functions as a signal-transducing GTPase. *J. Cell Biol.* 150, 145–154 (2000).
27. Enari, M., Ohmori, K., Kitabayashi, I. & Taya, Y. Requirement of clathrin heavy chain for p53-mediated transcription. *Genes Dev.* 20, 1087–1099 (2006).
28. Sharpless, N. E. & DePinho, R. A. Telomeres, stem cells, senescence, and cancer. *J. Clin. Invest.* 113, 160–168 (2004).
29. Sherley, J. L., Stadler, P. B. & Johnson, D. R. Expression of the wild-type p53 antioncogene induces guanine nucleotide-dependent stem cell division kinetics. *Proc. Natl Acad. Sci. USA* 92, 136–140 (1995).
30. Rambhatla, L., Rani-Mohan, S., Cheng, J. J. & Sherley, J. L. Immortal DNA strand cosegregation requires p53/IMPDH-dependent asymmetric self-renewal associated with adult stem cells. *Cancer Res.* 65, 3155–3161 (2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank K. Helin for the p53 and HDM2 reagents; L. Van Parijs for the pLL3.7 lentiviral vector; R. Bernard for the *p53* shRNA pSUPER vector; G. Matera for technical assistance; P. Maisonneuve and G. Goisis for statistical analysis; the Imaging Service at IEO; and the Real Time PCR Service at IFOM. This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro and MIUR to S.P. and P.P.D.F., from the European Community (VI Framework), The Ferrari Foundation, the Monzino Foundation and the CARIPLO Foundation to P.P.D.F., and from the G. Vollaro Foundation to S.P.

Author Contributions I.N.C., D.T., F.S.-N. and S.P. performed experimental work. P.N., V.G. and G.V. performed the clinical part of the work (patient selection, histology and data analysis of the patient's case collection). S.P. and P.P.D.F. planned and supervised the project, performed data analysis and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.P. (salvatore.pece@ifom-ieo-campus.it) or P.P.D.F. (pierpaolo.difiore@ifom-ieo-campus.it).

LETTERS

Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins

Ivan Ahel[1]*, Dragana Ahel[2]*, Takahiro Matsusaka[3], Allison J. Clark[2], Jonathon Pines[3], Simon J. Boulton[2] & Stephen C. West[1]

Post-translational modification (PTM) of proteins plays an important part in mediating protein interactions and/or the recruitment of specific protein targets[1,2]. PTM can be mediated by the addition of functional groups (for example, acetylation or phosphorylation), peptides (for example, ubiquitylation or sumoylation), or nucleotides (for example, poly(ADP-ribosyl)ation). Poly(ADP-ribosyl)ation often involves the addition of long chains of ADP-ribose units, linked by glycosidic ribose–ribose bonds[3], and is critical for a wide range of processes, including DNA repair, regulation of chromosome structure, transcriptional regulation, mitosis and apoptosis[4]. Here we identify a novel poly(ADP-ribose)-binding zinc finger (PBZ) motif in a number of eukaryotic proteins involved in the DNA damage response and checkpoint regulation. The PBZ motif is also required for post-translational poly(ADP-ribosyl)ation. We demonstrate interaction of poly(ADP-ribose) with this motif in two representative human proteins, APLF (aprataxin PNK-like factor) and CHFR (checkpoint protein with FHA and RING domains), and show that the actions of CHFR in the antephase checkpoint are abrogated by mutations in PBZ or by inhibition of poly(ADP-ribose) synthesis.

Damaged DNA and the mitotic apparatus (mitotic spindle, centromeres and centrosome) represent major sites of poly(ADP-ribose) accumulation[5]. The specific targeting of proteins to these sites is dependent on the recognition of poly(ADP-ribose) (PAR) by defined PAR-binding motifs or modules. Until now, only two such motifs have been described. One is found in proteins such as p53, histones and XRCC1, and is characterized by a 20 amino acid motif containing a basic residue-rich cluster and a pattern of hydrophobic amino acids interspersed with basic residues[6,7]; the second is a conserved ~190-residue domain known as the macro domain, and is found in the poly(ADP-ribose) polymerases PARP9, PARP14 and PARP15 (ref. 8).

CHFR is a ubiquitin ligase that functions in the antephase checkpoint by actively delaying passage into mitosis in response to microtubule poisons[9,10]. It is frequently mutated in human epithelial cancers[10], and CHFR-deficient mice develop spontaneous tumours[11,12], but a detailed understanding of its function is still emerging. Analysis of the primary sequence of CHFR revealed a conserved putative C2H2 zinc-finger motif at its carboxy terminus. Using homology and pattern searches, similar motifs were found in other eukaryotic DNA repair and checkpoint control proteins (Supplementary Fig. 1a, b). Similarities between a subset of these proteins have been noted[13–15], but the function of the motif was not elucidated.

The putative C2H2 zinc-finger is separated by a 6–8 amino acid spacer and has the consensus [K/R]xxCx[F/Y]GxxCxbbxxxxHxxx [F/Y]xH (Supplementary Fig. 1b). On the basis of the data that follows, the motif will be referred to as PBZ. The phylogenetic distribution of the PBZ motif is limited to eukaryotes, excluding yeast, and, as such, its occurrence coincides with the presence of poly(ADP-ribose) polymerases (PARPs). Because the majority of proteins containing PBZ motifs are either directly or indirectly associated with PAR metabolism, we analysed the PAR-binding ability of two human representatives, CHFR and APLF. APLF (C2 or f13) is an FHA-domain protein involved in the DNA damage response[13–15]. The modular structures of CHFR and APLF revealed one and two PBZ motifs, respectively (Fig. 1a). Additionally, *Caenorhabditis elegans* DNA ligase III, containing a single PBZ motif at its C terminus, was also analysed. Purified recombinant proteins were dot-blotted onto a nitrocellulose membrane and tested for their ability to bind ^{32}P-labelled poly(ADP-ribose). PAR binding was observed with all three proteins, and was resistant to extensive washing with 1 M salt (Fig. 1b, lanes 1–3). The interaction with PAR was equal to, or better than, that observed with XRCC1, which binds PAR with high affinity (lane 4)[6,15].

Mutation of the conserved cysteine residues in the single putative PBZ motif within CHFR (Fig. 1a, indicated in red) resulted in the inability of the CHFR*PBZ mutant to bind PAR (Fig. 1c, compare lanes 5 and 6). With APLF, PAR-binding was abolished by mutation of both putative zinc-finger motifs (APLF*PBZ), but not by mutation of a single motif (APLF*PBZ1 and APLF*PBZ2) (Fig. 1c, lanes 1–4). The tandem motifs of APLF, when purified as a recombinant glutathione *S*-transferase (GST)-tagged protein, exhibited PAR binding (Fig. 1c, lane 7).

Depletion of zinc, by incubation of wild-type CHFR and APLF with the metal-chelating agent EDTA, resulted in a severe reduction in the ability of each protein to bind PAR (Fig. 1d, lanes 2 and 5). Subsequent incubation with excess zinc, however, restored PAR-binding ability to CHFR (lane 6) and *C. elegans* DNA ligase III (data not shown), but not to APLF which was irreversibly inactivated (lane 3). We conclude that PAR binding is dependent on the presence of zinc, and define this newly identified motif as a PAR-binding zinc-finger or PBZ module. To our knowledge, this is the first description of a zinc finger that is involved in PAR binding.

Interactions between PAR and recombinant CHFR and APLF were further analysed by surface plasmon resonance (Fig. 1e). Wild-type CHFR and APLF bound PAR efficiently, and the kinetics of binding and dissociation are shown in Fig. 1e and Supplementary Table 1. The interactions of PAR with CHFR and APLF were significantly more stable than that observed with XRCC1. The binding of PAR by CHFR*PBZ or APLF*PBZ was not detectable (Fig. 1e).

To investigate whether APLF and CHFR were themselves substrates for poly(ADP-ribosyl)ation, they were incubated with

[1]Genetic Recombination and [2]DNA Damage Response Laboratories, Cancer Research UK London Research Institute, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, UK. [3]Wellcome Trust/Cancer Research UK Gurdon Institute, Tennis Court Road, Cambridge CB2 1QR, UK
*These authors contributed equally to this work

PARP1 in the presence of ^{32}P-labelled NAD (Fig. 1f). PARP1 poly(ADP-ribosyl)ated wild-type APLF and CHFR, as well as APLF*PBZ2 (lanes 2, 3 and 5), whereas the CHFR*PBZ and APLF*PBZ mutants remained unmodified (lanes 4 and 6). Thus, an intact PBZ motif is required for poly(ADP-ribosyl)ation.

Mutational analysis of the PBZ motif revealed that the conserved arginine preceding the zinc finger was required for PAR-binding in both APLF (APLF*R1) and CHFR (CHFR*R1) (Fig. 1g, lanes 6 and 12). Furthermore, mutations at residues following the second cysteine of PBZ compromised PAR binding to CHFR*R2, CHFR*Q, APLF*Y, APLF*R2 and APLF*R2K (lanes 2, 3, 8, and 10, and Supplementary Fig. 2). All APLF mutants deficient for PAR-binding were not poly(ADP-ribosyl)ated by PARP1 *in vitro* (Supplementary Fig. 2).

We next determined whether APLF and CHFR associate with PAR *in vivo*. When Flag-tagged APLF and CHFR proteins were transiently expressed in HEK293T cells, we found that the Flag pull downs of each protein contained PAR, as detected by western blotting (Fig. 2a, lanes 3 and 7). The PBZ-inactivating mutations severely reduced or abolished the associations of both APLF and CHFR with PAR (Fig. 2a, lanes 4 and 8, and Fig. 2b, lanes 3, 4, 6 and 7). Interestingly, PARP1 was present in both the APLF and the APLF*PBZ immunoprecipitates (Fig. 2a, lanes 3 and 4), but not in the CHFR pull downs (lanes 7 and 8). We also found that expression of green fluorescent protein (GFP)–APLF and GFP–CHFR fusion proteins in HEK293T cells resulted in the formation of distinct nuclear foci that co-localized with PAR (Fig. 2c), even in the absence of DNA damage. The co-localization of PAR with GFP–APLF and GFP–CHFR, but not with their PBZ mutant derivatives, indicates that the overexpressed wild-type proteins are poly(ADP-ribosyl)ated *in vivo*. We do not, however, rule out the possibility that the overexpressed proteins might recruit other poly(ADP-ribosyl)ated proteins leading to the observed signals. In control experiments, PAR failed to localize with GFP when expressed without CHFR or APLF (data not shown).

To examine the functional importance of the PBZ motif, we analysed its requirement for the CHFR-dependent antephase checkpoint in Ptk1 cells. Following treatment with microtubule poisons (such as colcemid), late G2 and prophase cells with an intact checkpoint delay entry to mitosis and decondense their chromosomes, while the nuclear envelope remains intact[9,17]. However, a CHFR deletion mutant lacking the amino-terminal FHA domain (CHFRΔFHA) acts as a *trans*-dominant inhibitor of endogenous CHFR function[10], and its expression abrogates the mitotic delay. We found that mutations of the cysteine residues in the PBZ motif of CHFRΔFHA

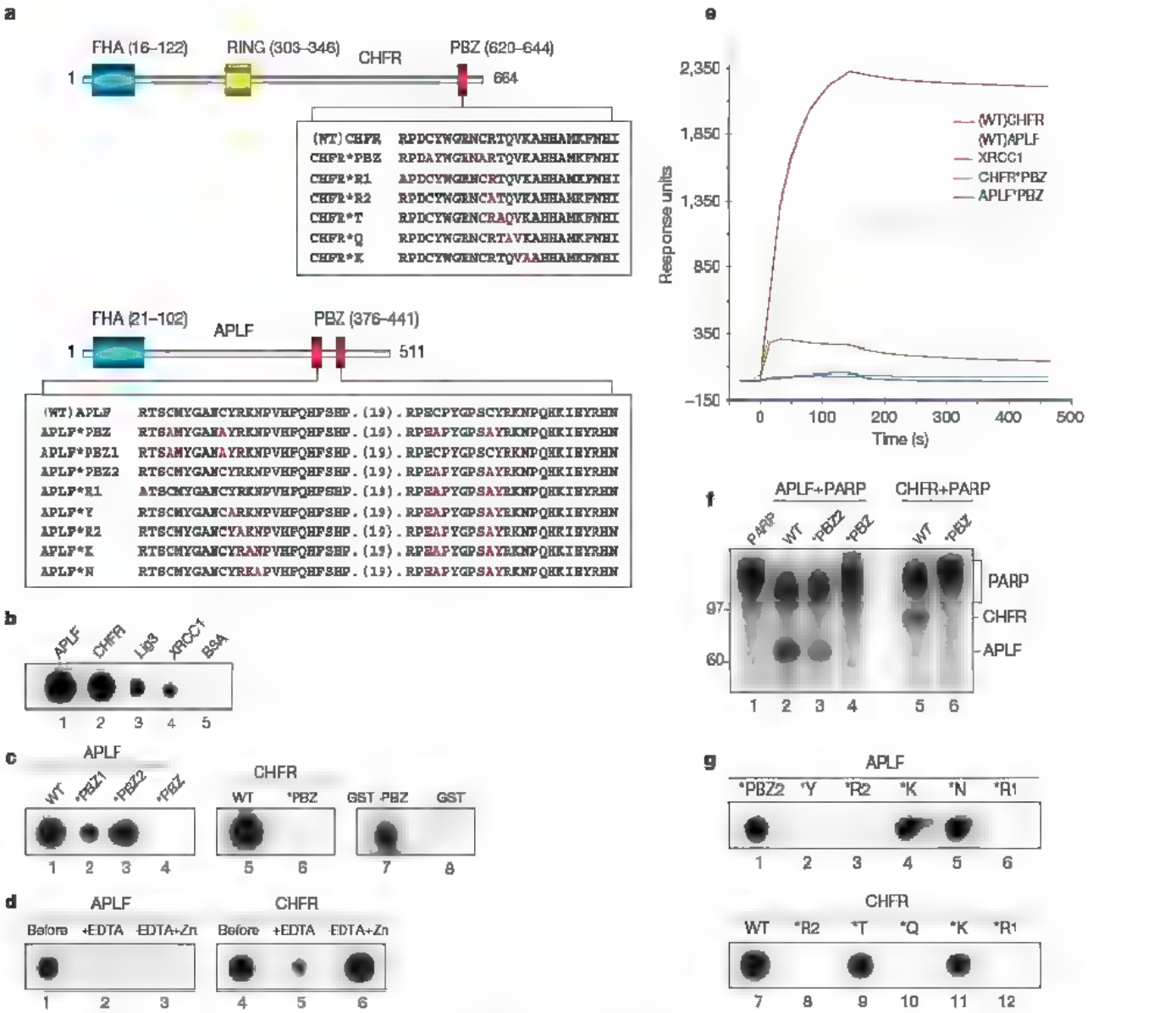


Figure 1 | PAR binding/modification mediated by the PBZ zinc finger motif. **a**, Schematic diagram of the PBZ motifs in CHFR and APLF. Mutations are indicated in red. **b**, PAR binding by APLF, CHFR and *C. elegans* DNA ligase III (lig3) as determined by dot-blot analysis. XRCC1 and BSA were used as controls. **c**, PAR-binding is abolished by mutations in the PBZ motif. WT, wild type. **d**, PAR-binding is dependent on zinc. **e**, Analysis of PAR binding by surface plasmon resonance. **f**, *In vitro* poly(ADP-ribosyl)ation of APLF and CHFR by PARP1. **g**, PAR binding by the APLF and CHFR PBZ mutants.

(CHFR*PBZΔFHA) abolished its ability to act as a *trans*-dominant inhibitor (Fig. 3a, b, and Supplementary videos 1 and 2). All prophase cells expressing CHFRΔFHA failed to exhibit an antephase checkpoint when exposed to colcemid (9 out of 9 cells), whereas those expressing CHFR*PBZΔFHA returned to interphase (6 out of 6 cells). Thus, the ability of CHFRΔFHA to act as a dominant negative relies on PBZ, which in turn suggests an involvement of the PBZ motif in regulating CHFR actions.

HeLa cells do not express CHFR and therefore lack an intact antephase checkpoint[9,18], leading us to use them as the parental cell line in a direct test of the physiological significance of PBZ in the antephase checkpoint. When HeLa cells were transfected with either wild-type or PBZ-mutated cyan fluorescent protein (CFP)-tagged CHFR and treated with colcemid, we found that expression of wild-type but not mutant CHFR restored the checkpoint defect, as indicated by a decrease in the mitotic index (Fig. 3c). Importantly, the auto-ubiquitylation activity of CHFR, both *in vivo* and *in vitro*, was unaffected by mutations in the PBZ motif (Fig. 2a, lanes 7 and 8, and Fig. 3d, lanes 7 and 11). Furthermore, auto-ubiquitylation of wild-type CHFR did not impair its PAR-binding potential (Fig. 3d, lanes 1 and 2). Hence, the disparity between CHFR and its PBZ-mutated variant in the antephase checkpoint most probably reflects differences in their PAR-related functions.

Finally, a direct link between PAR metabolism and the antephase checkpoint was established by treating Ptk1 cells with the specific PARP inhibitor KU-0058948[19]. We found that the PARP inhibitor compromised the ability of Ptk1 cells to delay nuclear envelope breakdown in response to microtubule poisons (Fig. 3e and Supplementary video 3). Instead, the majority of the treated cells continued into mitosis. These results show that inhibition of PAR synthesis compromises the antephase checkpoint, and demonstrate a connection between the PAR-related functions of PBZ and its requirement for CHFR checkpoint regulation.

In this work, we have defined a novel PAR-interaction motif present in a number of proteins associated with the DNA damage response and checkpoint regulation. Although two functionally equivalent domains have previously been reported, this is the first example of a zinc-dependent motif implicated in PAR binding and poly(ADP-ribosyl)ation. Zinc fingers were originally identified as nucleic acid recognition elements[20], but can also mediate protein–protein interactions[21]. Owing to its chemical composition, PAR may be considered as the third type of nucleic acid[3], a notion supported by the base stacking and hydrogen-bonding potential of its constituting ADP-ribose residues. Moreover, long PAR chains were postulated to adopt helical conformations, reminiscent of those found with DNA and RNA[22]. In light of this, the evolution of zinc-fingers into PAR-binding elements may seem a suitable consequence of diversification.

The use of the PBZ motif is widespread amongst eukaryotes, and is particularly prominent in *Dictyostelium discoideum* (Supplementary Fig. 1). The absence of the motif in organisms lacking PARP metabolism (such as prokaryotes and yeasts) may suggest the co-evolution of this motif with PARPs. Importantly, all the PBZ motifs identified in this study were found in proteins potentially regulated by poly(ADP-ribosyl)ation. The majority are DNA damage response proteins, including several PARPs, PARP-related proteins, Ku, Chk2, RAD17, APLF, and proteins involved in single-strand break

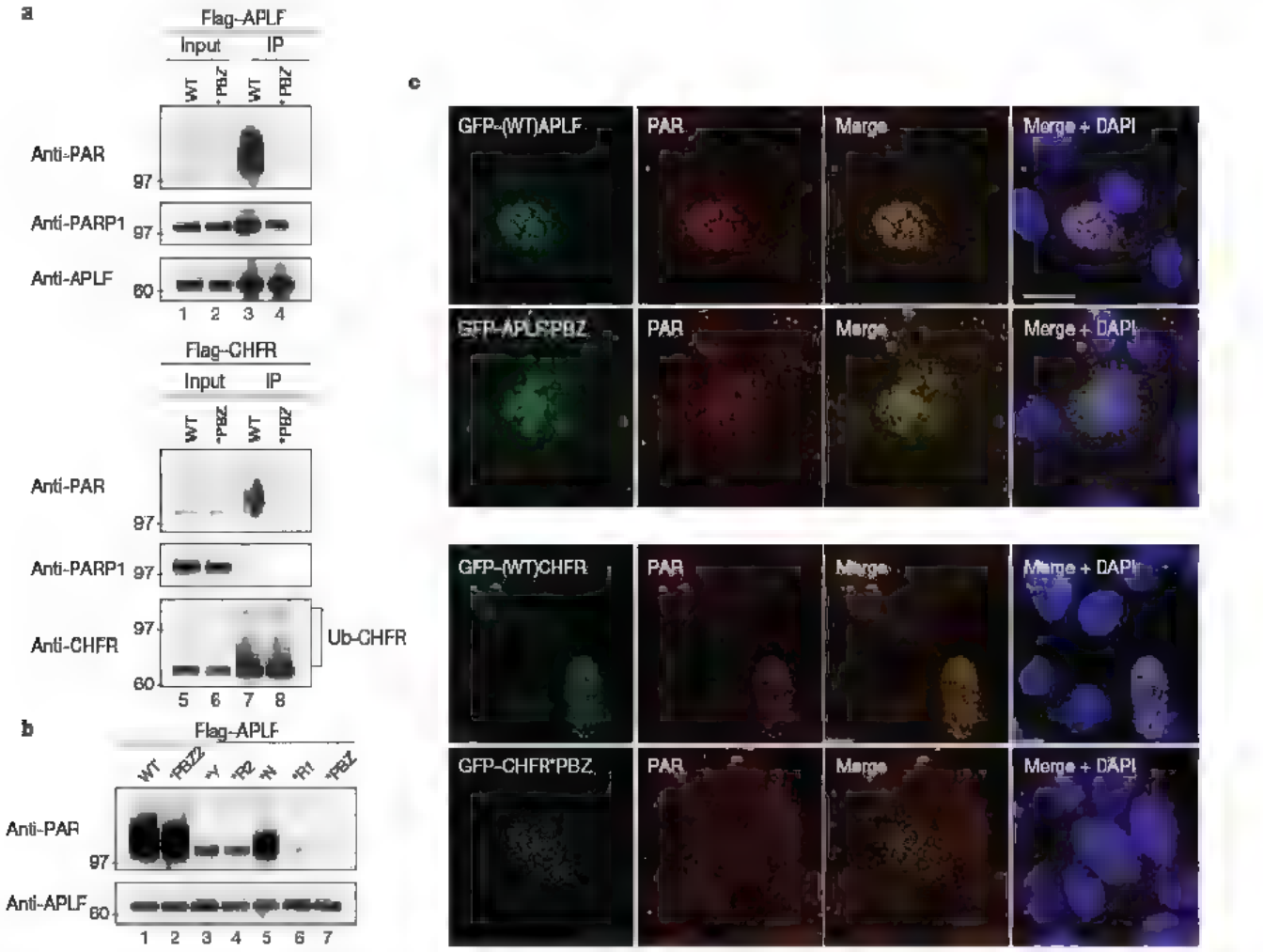
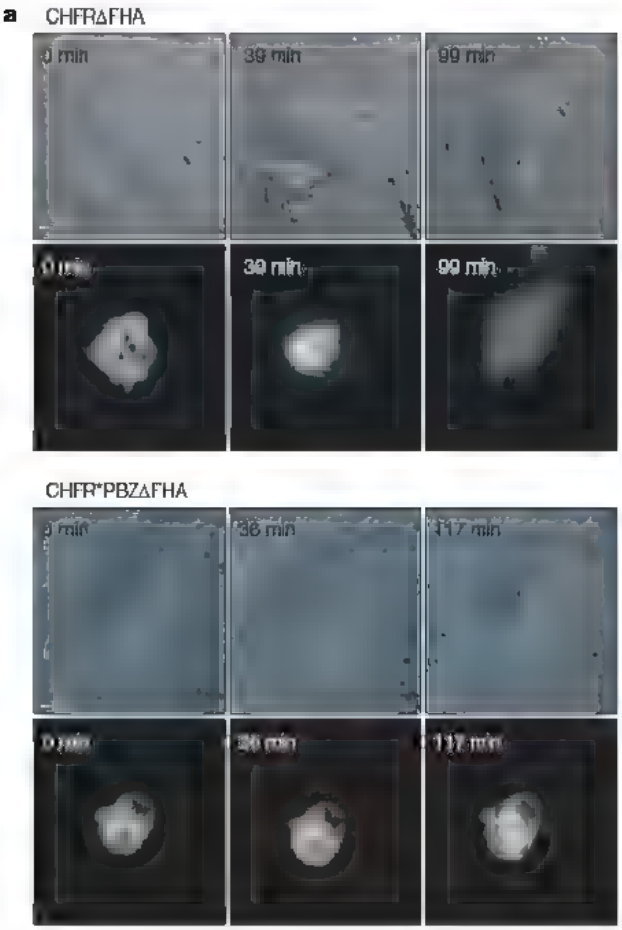
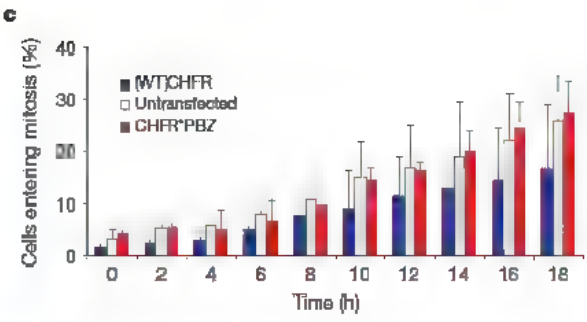


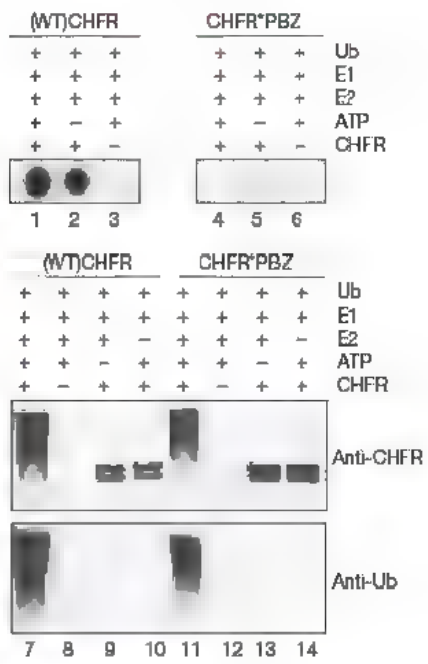
Figure 2 | Interactions of APLF and CHFR with PAR are mediated by the PBZ motif. **a**, Immunoprecipitation of Flag-tagged APLF and CHFR from HEK293T extracts. Inputs (10%) and Flag-precipitates were blotted using PAR, APLF and CHFR antibodies. Ubi-CHFR indicates ubiquitylated CHFR. **b**, Association of PAR with wild-type and PBZ-mutated Flag-tagged APLF. **c**, Co-localization of wild-type APLF and CHFR proteins with PAR in HEK293T cells transfected with GFP-tagged APLF and CHFR wild-type and mutant constructs. Scale bar, 10 μm.



b

	Return to interphase	Continue to mitosis	Arrest in prophase
CHFRΔFHA	0	4	5
CHFR*PBZΔFHA	6	0	0
(WT)CHFR	5	0	2
Untransfected	13	3	0





e

	Return to interphase	Continue to mitosis
KU-0058948	3	13
PBS	5	1

Figure 3 | Checkpoint functions, but not ubiquitylation, of CHFR are affected by PBZ mutations. **a**, Ptk1 cells expressing CFP-tagged CHFRΔFHA (top panels) or CHFR*PBZΔFHA (lower panels) were treated with 15 μM colcemid (0 min) and their behaviour was monitored by time-lapse DIC and fluorescence microscopy at 3-min intervals. Scale bar, 10 μm. **b**, Quantification of the data from **a**. **c**, HeLa cells expressing wild-type or mutant CHFR and untransfected cells were treated with colcemid and the mitotic indices (±s.d., $n = 3$) were determined at two-hourly intervals. **d**, Auto-ubiquitylation of CHFR does not impair PAR-binding. Wild-type and mutant CHFR were ubiquitylated *in vitro* and analysed for PAR binding (upper panels) or by western blotting (lower panels). **e**, Analysis of Ptk1 cells pre-treated with the PARP inhibitor KU-0058948 (1 μM), or PBS for 1 h, and challenged with 15 μM colcemid. Their behaviour was monitored by time-lapse DIC.

and base-excision repair (for example, tyrosyl-DNA phosphodiesterase, DNA ligase III and uracil DNA glycosylase). The modulation of DNA ligase III activity by PAR and interactions with poly(ADP-ribosyl)ated PARP1 have been described previously[23]. Similarly, Ku and PARP1 form a complex, the properties of which are changed on its poly(ADP-ribosyl)ation[24].

Using CHFR, we established the functional importance of the PBZ motif, demonstrating that specific PBZ-targeted mutations abrogate CHFR function in the antephase checkpoint and that treatment with a PARP inhibitor abolished this checkpoint in CHFR-proficient cells. Thus, PAR assumes a major role in modulating CHFR activity, and consequently in regulation of the antephase checkpoint in response to microtubule poisons. The physiological importance of the PBZ motif is further supported by observations that APLF localizes at sites of DNA damage, by a mechanism dependent on the region of APLF containing the PBZ motif and on PAR synthesis[13–15]. Given that APLF interacts directly with poly(ADP-ribosyl)ated PARP1, we propose that this association defines a role for APLF in DNA break repair.

In general, PAR modifications regulate a dynamic network of intermolecular associations. It has been estimated that PARP1-associated PAR constitutes the major fraction of PAR within the cell[25]. Consequently, automodified PARP1 is likely to attract proteins with PAR-binding motifs, the subsequent poly(ADP-ribosyl)ation of which may be a secondary effect that provides an additional level of regulation. This would be consistent with our results demonstrating efficient binding of PAR by APLF and CHFR, and the ability of these proteins to be poly(ADP-ribosyl)ated by PARP1. Collectively, these data define a novel poly(ADP-ribose)-binding zinc finger and indicate a mechanism by which cells use modification-dependent interactions to orchestrate the assembly of regulatory pathways.

METHODS SUMMARY

All proteins were purified after expression in *Escherichia coli*. Modification by PARP1 was carried out using a PARP activity assay kit (Trevigen). PAR binding was assessed in dot-blot assays and quantitated by Surface Plasmon Resonance using a BIACORE 3000. Off-rates were determined in the presence of PAR. Transient expression of Flag-tagged proteins in human embryonic kidney 293T cells allowed the co-immunoprecipitation of protein–PAR and

protein–protein complexes. Transiently expressed GFP-/CFP-tagged proteins and PAR-specific antibodies were used in immunofluorescence studies. CHFR checkpoints in Ptk1 and Hela cells treated with colcemid were analysed by time-lapse differential interference contrast (DIC) and fluorescence microscopy. *In vitro* CHFR auto-ubiquitylation was performed as described[25]. Detailed experimental procedures are found in Supplementary Information and Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 8 August; accepted 26 October 2007.

1. Walsh, C. T. *Posttranslational Modification of Proteins: Expanding Nature's Inventory* (Roberts and Co., Greenwood Village, Colorado, 2006)
2. Seet, B. T. *et al.* Reading protein modifications with interaction domains. *Nature Rev. Mol. Cell Biol.* 7, 473–483 (2006).
3. D'Amours, D. *et al.* Poly(ADP-ribosyl)ation reaction in the regulation of nuclear functions. *J. Biochem.* 342, 249–268 (1999).
4. Kim, M. Y., Zhang, T. & Kraus, W. L. Poly(ADP-ribosyl)ation by PARP-1 'PAR-laying' NAD^+ into a nuclear signal. *Genes Dev.* 19, 1951–1967 (2005).
5. Hassa, P. O. *et al.* Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? *Microbiol. Mol. Biol. Rev.* 70, 789–829 (2006).
6. Pleschke, J. M. *et al.* Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. *J. Biol. Chem.* 275, 40974–40980 (2000).
7. Gagne, J. P. *et al.* A proteomic approach to the identification of heterogeneous nuclear ribonucleoproteins as a new family of poly(ADP-ribose)-binding proteins. *Biochem. J.* 371, 331–340 (2003).
8. Karras, G. I. *et al.* The macro domain is an ADP-ribose binding module. *EMBO J.* 24, 1911–1920 (2005).
9. Matsusaka, T. & Pines, J. CHFR acts with the p38 stress kinases to block entry to mitosis in mammalian cells. *J. Cell Biol.* 166, 507–516 (2004).
10. Scolnick, D. M. & Halazonetis, T. D. CHFR defines a mitotic stress checkpoint that delays entry into metaphase. *Nature* 406, 430–435 (2000).
11. Kang, D. *et al.* The checkpoint protein CHFR is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G2 to M transition. *J. Cell Biol.* 156, 249–259 (2002).
12. Yu, X. *et al.* CHFR is required for tumour suppression and Aurora A regulation. *Nature Genet.* 37, 401–406 (2005).
13. Iles, N. *et al.* APLF (C2orf13) is a novel protein involved in the cellular response to chromosomal DNA strand breaks. *Mol. Cell. Biol.* 27, 3793–3803 (2007).
14. Kanno, S.-I. *et al.* A novel human AP endonuclease with conserved zinc-finger-like motifs involved in DNA strand break responses. *EMBO J.* 26, 2094–2103 (2007).
15. Bekker-Jensen, S. *et al.* Human XIP1 (C2orf13) is a novel regulator of cellular responses to DNA strand breaks. *J. Biol. Chem.* 282, 19638–19643 (2007).
16. Masson, M. *et al.* XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA-damage. *Mol. Cell. Biol.* 18, 3563–3571 (1998).
17. Rieder, C. L. & Cole, R. Entry into mitosis in vertebrate somatic cells is guarded by a chromosome damage checkpoint that reverses the cell cycle when triggered during early but not late prophase. *J. Cell Biol.* 142, 1013–1022 (1998).
18. Rieder, C. L. & Cole, R. Microscopy-induced radiation damage, microtubules, and progression through the terminal stage of G2 (prophase) in vertebrate somatic cells. *Cold Spring Harb. Symp. Quant. Biol.* 65, 369–376 (2000).
19. Farmer, H. *et al.* Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434, 917–921 (2005).
20. Miller, J., McLachlan, A. D. & Klug, A. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. *EMBO J.* 4, 1609–1614 (1985).
21. Garnsjaeger, R. *et al.* Sticky fingers: zinc fingers as protein recognition motifs. *Trends Biochem. Sci.* 32, 63–70 (2007).
22. Minaga, T. & Kun, E. Probable helical conformation of poly(ADP-ribose). The effect of cations on spectral properties. *J. Biol. Chem.* 258, 5726–5730 (1983).
23. Leppard, J. B. *et al.* Physical and functional interaction between DNA ligase IIIα and poly(ADP-ribose) polymerase 1 in DNA single-strand break repair. *Mol. Cell. Biol.* 23, 5919–5927 (2003).
24. Galande, S. & Kohwi-Shigematsu, T. Poly(ADP-ribose) polymerase and Ku autoantigen form a complex and synergistically bind to matrix attachment sequences. *J. Biol. Chem.* 274, 20521–20528 (1999).
25. Burkle, A. Poly(ADP-ribose). The most elaborate metabolite of NAD^+. *FEBS J.* 272, 4576–4589 (2005).
26. Bothos, J. *et al.* The CHFR checkpoint protein functions with Ubc13-Mms2 to form Lys63-linked polyubiquitin chains. *Oncogene* 22, 7101–7107 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Lindahl (LRI, CRUK) for XRCC1, G. Smith for the PARP inhibitor KU-0058948, and J. Gannon for assistance with the Biacore. This work was supported by Cancer Research UK, the EU DNA Repair Consortium and the Louis-Jeantet Foundation. I.A. and D.A. are supported by EMBO fellowships.

Author Contributions I.A. and D.A. discovered the PBZ motif and performed most of the experiments. A.J.C. carried out supporting analyses. T.M. and J.P. defined the role of PBZ in the antephase checkpoint. S.J.B. and S.C.W. are joint senior authors who managed the project and helped write the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.J.B. (simon.boulton@cancer.org.uk) or S.C.W. (stephen.west@cancer.org.uk)

LETTERS

Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels

Shota Atsumi[1], Taizo Hanai[1] & James C. Liao[1,2]

Global energy and environmental problems have stimulated increased efforts towards synthesizing biofuels from renewable resources[1–3]. Compared to the traditional biofuel, ethanol, higher alcohols offer advantages as gasoline substitutes because of their higher energy density and lower hygroscopicity. In addition, branched-chain alcohols have higher octane numbers compared with their straight-chain counterparts. However, these alcohols cannot be synthesized economically using native organisms. Here we present a metabolic engineering approach using *Escherichia coli* to produce higher alcohols including isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol from glucose, a renewable carbon source. This strategy uses the host's highly active amino acid biosynthetic pathway and diverts its 2-keto acid intermediates for alcohol synthesis. In particular, we have achieved high-yield, high-specificity production of isobutanol from glucose. The strategy enables the exploration of biofuels beyond those naturally accumulated to high quantities in microbial fermentation.

Ethanol is not an ideal fuel because it has a lower energy density than gasoline, and its hygroscopicity poses a problem for storage and distribution. Higher alcohols (C4 and C5), on the other hand, have energy densities closer to gasoline, are not hygroscopic, and are less volatile compared with ethanol. Except for 1-butanol[1,2], none of the C4 and C5 alcohols has been produced from a renewable source in a yield high enough to be considered as a gasoline substitute. No microorganisms have been identified to produce, from glucose, higher alcohols such as isobutanol, 2-methyl-1-butanol or 3-methyl-1-butanol to industrially relevant quantities, although small amounts have been identified as microbial by-products[4–8].

Here, we devised a synthetic approach to produce the above-mentioned longer chain alcohols as next-generation biofuels. This strategy was implemented in *E. coli*, although other user friendly hosts such as *Saccharomyces cerevisiae* are readily applicable. These host organisms have fast growth rates and are facultative anaerobes, allowing for a flexible and economical process design for large-scale production[3,9,10]. However, importing and the expression of non-native pathways may lead to metabolic imbalance, whereas the accumulation of the heterologous metabolites may cause cytotoxicity[11–13]. To achieve high productivity of the target foreign products, it is desirable to seek pathways that are compatible to the host. Therefore, we took advantage of the existing metabolic capability in *E. coli* and the broad substrate range of the last two steps in the Ehrlich pathway[14] for 2-keto acid degradation from other organisms.

2-Keto acids are intermediates in amino acid biosynthesis pathways. These metabolites can be converted to aldehydes by broad-substrate-range 2-keto-acid decarboxylases (KDCs) and then to alcohols by alcohol dehydrogenases (ADHs). Using this strategy, only two non-native steps were needed to produce biofuels by shunting intermediates from amino acid biosynthesis pathways to alcohol production (Fig. 1a). Amino acid biosynthesis pathways produce various 2-keto acids (Fig. 1b). In this work, six different 2-keto acids for alcohol production were used. The isoleucine biosynthesis pathway generates 2-ketobutyrate and 2-keto-3-methyl-valerate, which can be converted to 1-propanol and 2-methyl-1-butanol, respectively. The valine biosynthesis pathway produces 2-keto-isovalerate, which is the precursor for isobutanol. The leucine biosynthesis pathway generates 2-keto-4-methyl-pentanoate, which is the substrate for 3-methyl-1-butanol. The phenylalanine biosynthesis pathway produces phenylpyruvate, which can lead to 2-phenylethanol. The norvaline biosynthesis pathway, which is a side-reaction of the leucine biosynthesis, produces a substrate for 1-butanol, 2-ketovalerate.

A critical enzyme in this alcohol production strategy is KDC, which is common in plants, yeasts and fungi but less so in bacteria[15]. The aldehydes produced can then be converted to alcohols by an ADH, which is commonly found in many organisms. Some of the KDCs have broad substrate ranges, whereas others are more specific. To test the capability of the endogenous 2-keto acids as a substrate for KDC in *E. coli*, five KDCs (Pdc6 (ref. 16), Aro10 (ref. 17), Thi3 (ref. 5) from *S. cerevisiae*, Kivd from *Lactococcus lactis*[18], and Pdc from *Clostridium acetobutylicum*) with alcohol dehydrogenase 2 (Adh2) of *S. cerevisiae*[19] were overexpressed. *E. coli* cultures expressing these foreign genes were grown in a minimal media with 0.2 M glucose. Gas chromatography–mass spectrometry (GC–MS) analysis (Table 1) revealed that the strains expressing either Kivd or Aro10 produced all of the expected alcohols. *S. cerevisiae* Pdc6 and *C. acetobutylicum* Pdc were not as versatile, whereas *S. cerevisiae* Thi3 did not have any expected activity. In all of these cases, aldehydes were detected only in trace amounts, indicating sufficient activity of Adh2. These results demonstrate that Kivd is the most active and versatile decarboxylase tested and, therefore, suited for our objectives. Furthermore, addition of various 2-keto acids (Table 2) to the *E. coli* culture expressing Kivd confirmed the specific production of the corresponding alcohols by 2- to 23-fold. The supply of 2-keto acids also decreased the production of the other alcohols markedly. These results indicate that increasing the flux to the 2-keto acids could improve both the productivity and specificity of production of the alcohols.

The existing *E. coli* metabolic pathways were then genetically modified to increase the production of the specific 2-keto acid so that the desired alcohol could be produced. To produce isobutanol, the *ilvIHCD* genes under the control of the $P_L lacO_1$ (ref. 20) promoter on a plasmid were overexpressed to enhance 2-ketoisovalerate biosynthesis (Fig. 1b). The amplified *ilv* pathway was then combined with the alcohol producing pathway (Kivd and Adh2) to achieve isobutanol production. As a result of the *ilvIHCD* overexpression, the strain produced 23 mM isobutanol, which is a ~5-fold increase over the strain without *ilvIHCD* overexpression (Fig. 2a and

[1]Department of Chemical and Biomolecular Engineering, [2]UCLA-DOE Institute for Genomics and Proteomics, University of California, Los Angeles, 5531 Boelter Hall, 420 Westwood Plaza, Los Angeles, California 90095, USA

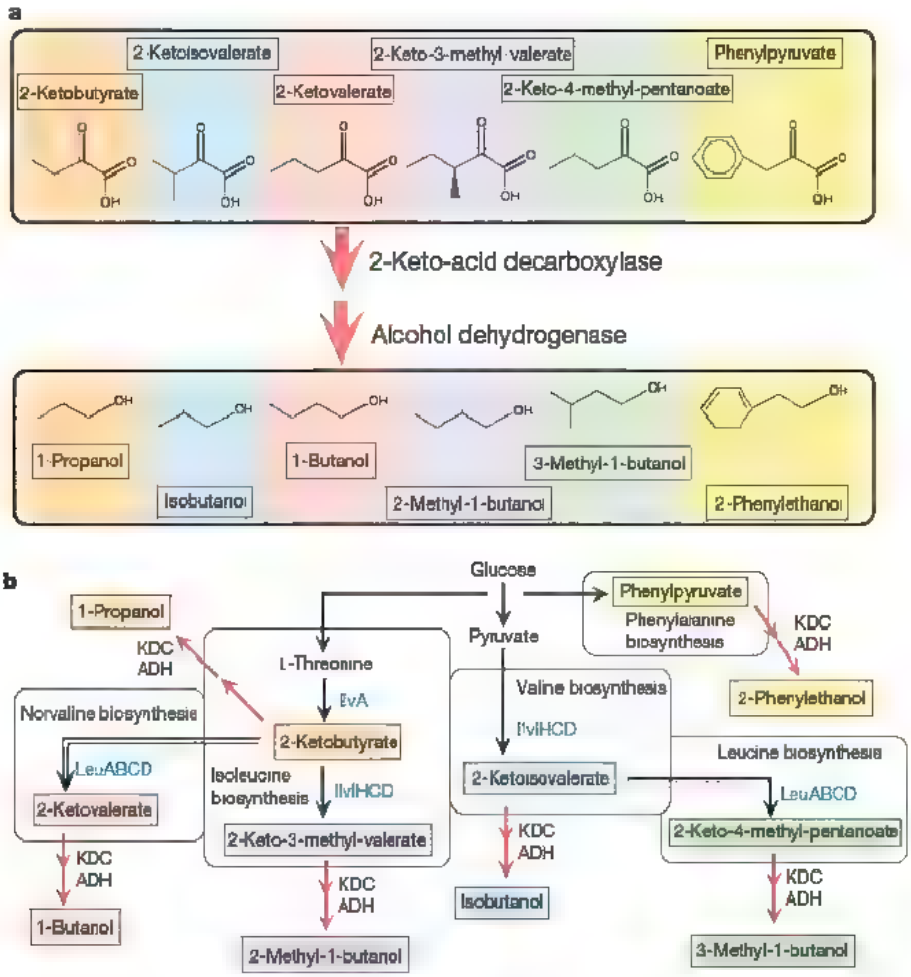


Figure 1 | Production of higher alcohols through the synthetic non-fermentative pathways. **a**, Various 2-keto acid precursors lead to corresponding alcohols through 2-ketoacid decarboxylase and alcohol dehydrogenase **b**, The synthetic networks for the non-fermentative alcohol production in engineered *E. coli*. Red arrows represent the 2-keto acid decarboxylation and reduction pathway. Blue enzyme names represent amino acid biosynthesis pathways. The double lines represent a side pathway leading to norvaline and 1-butanol biosynthesis.

Supplementary Table 3). These results demonstrate that the synthetic pathway was functional and capable of supplying the 2-ketoisovalerate required for the efficient production of isobutanol. To increase further the isobutanol production, genes that contribute to by-product formation, including *adhE*, *ldhA*, *frdAB*, *fnr* and *pta*, were deleted. These deletions could increase the level of pyruvate available for the *ilvIHCD* pathway. Indeed, this strain produced 30 mM isobutanol, indicating that these deletions were beneficial for isobutanol production. In addition, this strain converted glucose to isobutanol with a yield of 0.21 g of isobutanol per gram of glucose between 16 h and 24 h (Fig. 2a, right panel)

To improve isobutanol production further, the *alsS* gene from *Bacillus subtilis* was used instead of *ilvIH* of *E. coli*. AlsS of *B. subtilis* has high affinity for pyruvate, whereas *E. coli* IlvIH has higher preference for 2-ketobutyrate[21]. The strain with the *alsS* pathway produced ~50 mM of isobutanol, which is a ~1.7-fold increase over the strain using IlvIH (Supplementary Fig. 1). In addition, *pflB* was deleted to decrease further the competition for pyruvate. The combined effects of these manipulations led to ~300 mM (22 g l^{-1}) of isobutanol under micro-aerobic conditions (Fig. 2b, left panel and Supplementary Fig. 2). In this experiment, 0.5% yeast extract was supplied in the medium to obtain higher cell density. As a control, this strain produced a negligible amount of isobutanol with 0.5% yeast extract without glucose (Supplementary Fig. 3). The yield reached 0.35 (g isobutanol per g glucose) between 40 h and 112 h (Fig. 2b, right panel), which is 86% of the theoretical maximum. This result demonstrates the potential of this strategy, as high-yield production was achieved even without detailed optimization of the pathways and production conditions.

To demonstrate the generality of this approach, the same strategy was also applied to 1-butanol production. Some clostridial species produce 1-butanol during fermentative growth and many of the enzymes in this pathway are oxygen-sensitive and CoA-dependent[22]. We found that by overexpressing Kivd or Aro10 in *E. coli*, which does not have the

Table 1 | Alcohol production with KDC and ADH in *E. coli*

Product (μM)	KDC/plasmid				
	Kivd/pSA55	Aro10/pSA56	Pdc6/pSA49	Thi3/pSA57	Pdc (*C. acetobutylicum*)/pSA58
1-Propanol	520	290	125	ND	ND
Isobutanol	5,242	2,094	260	ND	75
1-Butanol	220	95	ND	ND	ND
2-Methyl-1-butanol	766	652	56	ND	ND
3-Methyl-1-butanol	1,495	1,099	92	ND	ND
2-Phenylethanol	324	469	ND	ND	175

The strain was JCL16 with various *kdc* genes and *S. cerevisiae ADH2* expressed from plasmids. Culture was grown in M9 medium with 0.2 M glucose plus 0.1 mM IPTG at 30 °C for 40 h. These products were identified by GC–MS and quantified by GC–FID (see Methods). ND, not detectable

Table 2 | Alcohol production with the supply of 2-keto acids

Product (μM)	2-Ketobutyrate	2-Keto-isovalerate	2-Ketovalerate	2-Keto-3-methyl-valerate	2-Keto-4-methyl-pentanoate	Phenylpyruvate
1-Propanol	2,138	ND	ND	ND	ND	8
Isobutanol	98	10,016	ND	ND	ND	64
1-Butanol	492	ND	3,926	ND	ND	23
2-Methyl-1-butanol	1,315	ND	ND	5,284	ND	ND
3-Methyl-1-butanol	ND	ND	52	ND	3,756	105
2-Phenylethanol	26	109	66	ND	ND	7,269

Strains and culture conditions are the same as described in Table 1. A total of 8 g l^{-1} of 2-keto acids was added, except for 2-ketovalerate, where 1 g l^{-1} was added because of its toxicity. ND, not detectable.

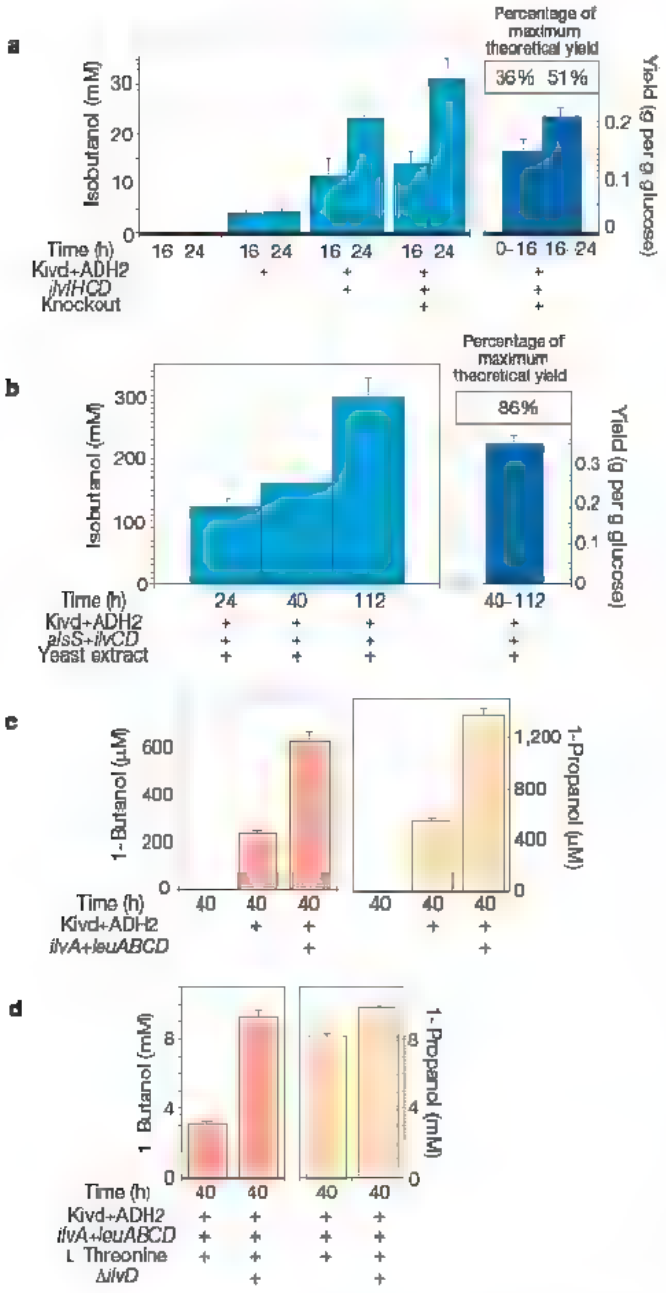


Figure 2 | Summary of results for isobutanol and 1-butanol production in *E. coli*. The cells were grown in M9 medium containing 36 g l^{-1} glucose in shake flasks at 30 °C with or without other nutrients indicated, and induced with 0.1 mM IPTG. Overexpressed genes and nutrient supplementation are indicated below the axis. Error bars indicate s.d. **a**, Left panel, isobutanol production, right panel, isobutanol yield per g of glucose. The theoretical maximum yield of isobutanol is 0.41 g g^{-1}. Knockout, Δ*adh*, Δ*ldh*, Δ*frd*, Δ*fnr* and Δ*pta*. **b**, Isobutanol production with *B. subtilis alsS* and yeast extract (5 g l^{-1}) supplementation to increase cell density. The host is JCL260. Detailed results are shown in Supplementary Fig. 2. **c**, **d**, Left panel, 1-butanol production; right panel, 1-propanol production in the same strain. The host strain is JCL16, with or without Δ*ilvD*. L-Threonine, L-threonine (8 g l^{-1}) supplementation.

1-butanol fermentative pathway, the cell produced a small amount of 1-butanol (Table 1) from glucose in a non-fermentative growth, indicating the existence of a corresponding 2-keto acid precursor, 2-ketovalerate. Unfortunately, 2-ketovalerate is not a common metabolite in *E. coli*. To increase the amount of synthesized 2-ketovalerate, we took advantage of the broad substrate specificity of the *leuABCD* pathway, the natural substrate of which is 2-ketoisovalerate (Fig. 1b). By using a smaller substrate, 2-ketobutyrate, which has one less methyl group than 2-ketoisovalerate (Fig. 1a), we attempted to synthesize 2-ketovalerate in a manner similar to the steps used in leucine biosynthesis[23]. 2-Ketobutyrate can be generated from L-threonine by the threonine dehydratase, encoded by the *ilvA* gene[24], or from an alternative pathway identified in *Leptospira interrogans*[25] and *Methanocaldococcus jannaschii*[26]. In the latter pathway, 2-ketobutyrate is generated from citramalate by the enzymes isopropylmalate isomerase (LeuCD) and β-isopropylmalate dehydrogenase (LeuB)[27].

Therefore, to produce 1-butanol, the operon encoding the *ilvA–leuABCD* pathway under the control of $P_L$lacO1 (ref. 20) was constructed. It was found that the strain with the *ilvA–leuABCD* pathway produced 0.6 mM 1-butanol, which is a ~3-fold increase compared with the strain without overexpression of this pathway (Fig. 2c and Supplementary Table 4). When the media was supplemented with 8 g l^{-1} L-threonine, a marked increase of 1-butanol production to 3.2 mM was observed, suggesting that 2-ketovalerate could be produced from L-threonine by means of an IlvA-mediated reaction (Fig. 2d).

To improve 1-butanol production further, the *ilvD* gene was deleted. This gene encodes dihydroxy-acid dehydratase[28], an enzyme that produces both 2-ketoisovalerate (a precursor for leucine and valine) and 2-keto-3-methyl-valerate (a precursor for isoleucine). This deletion could be beneficial for two reasons. First, the deletion of *ilvD* eliminates the native substrate, 2-ketoisovalerate, for the *leuABCD* pathway, thus reducing inhibition by the competitive substrate. Second, the deletion of *ilvD* eliminates competing substrates for Kivd: 2-keto-3-methyl-valerate and 2-keto-4-methyl-pentanoate. As expected, deletion of *ilvD* improved 1-butanol production (Fig. 2d).

Because strains of *E. coli* that hyperproduce L-threonine have been developed[29] for commercial production, it would be straightforward to modify a threonine producing strain with the above strategy for 1-butanol production. For further improvement, it would be necessary to increase the activity of the *leuABCD* pathway towards the non-native substrate, 2-ketobutyrate, and to raise the specificity of Kivd for 2-ketovalerate. Because 2-ketobutyrate is also the substrate for 1-propanol (Fig. 1b), increasing 2-ketobutyrate availability also enhances the production of 1-propanol (Fig. 2c, d, right). Therefore, increasing the LeuABCD activity and the specificity of KDC would be crucial for high-efficiency 1-butanol production.

Non-native hosts such as *E. coli* lack tolerance to high alcohols. Isobutanol is slightly less toxic to microorganisms than 1-butanol. The native 1-butanol producers can tolerate concentrations of 1-butanol up to about 2% (w/v) (ref. 1). To show the potential for improving tolerance, we conducted serial transfer of cultures to enrich for isobutanol-tolerant strains. We found that a wild-type *E. coli* strain (JCL16) was inhibited by 1.5% (w/v) isobutanol. However, after only five rounds of culture transfer with increasing isobutanol

concentrations, mutants were found to grow in the presence of 2% (w/v) isobutanol (Supplementary Fig. 4). This level of solvent tolerance is comparable or better than native producers of 1-butanol[1], suggesting that *E. coli* can adapt to high concentrations of long-chain alcohols. Other strategies such as global transcription machinery engineering can be used for further improvement of tolerance[30].

The strategy described above opens up an unexplored frontier for biofuels production, both in *E. coli* and in other microorganisms. This strategy takes advantage of the well-developed amino acid production technology, and channels the amino acid intermediates to the 2-keto acid degradation pathway for alcohol production. The strategy avoids CoA-mediated chemistry, which is commonly used in alcohol production in native organisms, and enables the synthesis of other higher and complex alcohols on large scales. Specific strategies for producing other alcohols can be readily devised based on the synthetic pathways and metabolic physiology. These strategies can also be implemented in yeast or other industrial microorganisms. In the case of isobutanol production, the complete pathway is CoA-independent and requires only pyruvate as a precursor. This feature avoids the mitochondria compartmentalization issue of acetyl-CoA when implementing the strategy in yeast.

METHODS SUMMARY

Strains and plasmids. The JCL16 strain is BW25113 (*rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$) with F′ transduced from XL-1 blue to supply *lacI*^{q}. JCL88 is JCL16 with Δ*adh*, Δ*ldh*, Δ*frd*, Δ*fnr* and Δ*pta*. JCL260 is the same as JCL88 but with Δ*pflB*. A list of the strains used is given in Supplementary Table 1. Construction of plasmids is described in Methods, and the primers used are listed in Supplementary Table 2.

Medium and cultivation. Unless stated otherwise, M9 medium containing 0.2 M glucose and 1,000th dilution of Trace Metal Mix A5 (2.86 g H_3BO_3, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}7H_2O$, 0.39 g $Na_2MoO_4{\cdot}2H_2O$, 0.079 g $CuSO_4{\cdot}5H_2O$, 49.4 mg $Co(NO_3)_2{\cdot}6H_2O$ per litre water) was used for cell growth. Ampicillin (100 μg ml^{-1}) and kanamycin (30 μg ml^{-1}) were added as appropriate. L-Valine (35 μg ml^{-1}), L-isoleucine (39.5 μg ml^{-1}) and L-leucine (39.5 μg ml^{-1}) were used to culture strains with Δ*ilvD*. Pre-culture in test tubes containing 3 ml of medium was performed at 37 °C overnight on a rotary shaker (250 r.p.m.). Overnight culture was diluted 1,100 into 20 ml of fresh medium in a 250-ml conical flask. For Fig. 2b, 250-ml screw-cap conical flasks were used. Cells were grown to an optical density at 600 nm of 0.8 at 37 °C, followed by adding 0.1 mM isopropyl-β-D-thiogalactoside (IPTG). For 1-butanol production (Fig. 2c, d), 8 g l^{-1} L-threonine was added together with IPTG. Cultivation was performed at 30 °C on a rotary shaker (250 r.p.m.). Gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionization detector (GC–FID) analyses are described in Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 4 May; accepted 2 November 2007.

1. Lin, Y. L. & Blaschek, H. P. Butanol production by a butanol-tolerant strain of *Clostridium acetobutylicum* in extruded corn broth. *Appl. Environ. Microbiol.* 45, 966–973 (1983)
2. Nair, R. V., Bennett, G. N. & Papoutsakis, E. T. Molecular characterization of an aldehyde/alcohol dehydrogenase gene from *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 176, 871–885 (1994).
3. Ingram, L. O. *et al.* Enteric bacterial catalysts for fuel ethanol production. *Biotechnol. Prog.* 15, 855–866 (1999).
4. Sentheshanuganathan, S. The mechanism of the formation of higher alcohols from amino acids by *Saccharomyces cerevisiae*. *Biochem. J.* 74, 568–576 (1960)
5. Dickinson, J. R. *et al.* A ^{13}C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 272, 26871–26878 (1997)
6. Dickinson, J. R., Harrison, S. J. & Hewlins, M. J. An investigation of the metabolism of valine to isobutyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273, 25751–25756 (1998)
7. Dickinson, J. R., Harrison, S. J., Dickinson, J. A. & Hewlins, M. J. An investigation of the metabolism of isoleucine to active Amyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 275, 10937–10942 (2000)
8. Dickinson, J. R. *et al.* The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 278, 8028–8034 (2003)
9. Farmer, W. R. & Liao, J. C. Improving lycopene production in *Escherichia coli* by engineering metabolic control. *Nature Biotechnol.* 18, 533–537 (2000).
10. Khosla, C. & Keasling, J. D. Metabolic engineering for drug discovery and development. *Nature Rev. Drug Discov.* 2, 1019–1025 (2003).
11. Barbirato, F., Grivet, J. P., Soucaille, P. & Bories, A. 3-Hydroxypropionaldehyde, an inhibitory metabolite of glycerol fermentation to 1,3-propanediol by enterobacterial species. *Appl. Environ. Microbiol.* 62, 1448–1451 (1996)
12. Zhu, M. M., Lawman, P. D. & Cameron, D. C. Improving 1,3-propanediol production from glycerol in a metabolically engineered *Escherichia coli* by reducing accumulation of sn-glycerol-3-phosphate. *Biotechnol. Prog.* 18, 694–699 (2002)
13. Pitera, D. J., Paddon, C. J., Newman, J. D. & Keasling, J. D. Balancing a heterologous mevalonate pathway for improved isoprenoid production in *Escherichia coli*. *Metab. Eng.* 9, 193–207 (2007)
14. Sentheshanmuganathan, S. & Elsden, S. R. The mechanism of the formation of tyrosol by *Saccharomyces cerevisiae*. *Biochem. J.* 69, 210–218 (1958).
15. Konig, S. Subunit structure, function and organisation of pyruvate decarboxylases from various organisms. *Biochim. Biophys. Acta* 1385, 271–286 (1998).
16. Hohmann, S. Characterization of PDC6, a third structural gene for pyruvate decarboxylase in *Saccharomyces cerevisiae*. *J. Bacteriol.* 173, 7963–7969 (1991)
17. Vuralhan, Z., Morais, M. A., Tai, S. L., Piper, M. D. & Pronk, J. T. Identification and characterization of phenylpyruvate decarboxylase genes in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 69, 4534–4541 (2003).
18. de la Plaza, M., Fernandez de Palencia, P., Pelaez, C. & Requena, T. Biochemical and molecular characterization of α-ketoisovalerate decarboxylase, an enzyme involved in the formation of aldehydes from amino acids by *Lactococcus lactis*. *FEMS Microbiol. Lett.* 238, 367–374 (2004)
19. Russell, D. W., Smith, M., Williamson, V. M. & Young, E. T. Nucleotide sequence of the yeast alcohol dehydrogenase II gene. *J. Biol. Chem.* 258, 2674–2682 (1983)
20. Lutz, R. & Bujard, H. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I_1–I_2 regulatory elements. *Nucleic Acids Res.* 25, 1203–1210 (1997).
21. Gollop, N., Damri, B., Chipman, D. M. & Barak, Z. Physiological implications of the substrate specificities of acetohydroxy acid synthases from varied organisms. *J. Bacteriol.* 172, 3444–3449 (1990)
22. Woods, D. R. The genetic engineering of microbial solvent production. *Trends Biotechnol.* 13, 259–264 (1995).
23. Bogosian, G. *et al.* Biosynthesis and incorporation into protein of norleucine by *Escherichia coli*. *J. Biol. Chem.* 264, 531–539 (1989).
24. Calhoun, D. H., Rimerman, R. A. & Hatfield, G. W. Threonine deaminase from *Escherichia coli*. I. Purification and properties. *J. Biol. Chem.* 248, 3511–3516 (1973)
25. Charon, N. W., Johnson, R. C. & Peterson, D. Amino acid biosynthesis in the spirochete *Leptospira*: evidence for a novel pathway of isoleucine biosynthesis. *J. Bacteriol.* 117, 203–211 (1974).
26. Howell, D. M., Xu, H. & White, R. H. (*R*)-citramalate synthase in methanogenic archaea. *J. Bacteriol.* 181, 331–333 (1999)
27. Xu, H. *et al.* Isoleucine biosynthesis in *Leptospira interrogans serotype lai* strain 56601 proceeds via a threonine-independent pathway. *J. Bacteriol.* 186, 5400–5409 (2004)
28. Flint, D. H., Emptage, M. H., Finnegan, M. G., Fu, W. & Johnson, M. K. The role and properties of the iron-sulfur cluster in *Escherichia coli* dihydroxy-acid dehydratase. *J. Biol. Chem.* 268, 14732–14742 (1993)
29. Miwa, K. *et al.* Construction of L-threonine overproducing strains of *Escherichia coli* K-12 using recombinant DNA techniques. *Agric. Biol. Chem.* 47, 2329–2334 (1983).
30. Alper, H., Moxley, J., Nevoigt, E., Fink, G. R. & Stephanopoulos, G. Engineering yeast transcription machinery for improved ethanol tolerance and production. *Science* 314, 1565–1568 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was partially supported by UCLA-DOE Institute for Genomics and Proteomics. We are grateful to H. Bujard for plasmids, and members of the Liao laboratory for discussion and comments on the manuscript.

Author Contributions S.A. and J.C.L. designed experiments; S.A. and T.H. performed the experiments; S.A. and J.C.L. analysed the data, and S.A. and J.C.L. wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to J.C.L. (liaoj@seas.ucla.edu).

LETTERS

Distinct domains of tRNA synthetase recognize the same base pair

Kirk Beebe[1]*, Marissa Mock[1]*, Eve Merriman[1] & Paul Schimmel[1]

Synthesis of proteins containing errors (mistranslation) is prevented by aminoacyl transfer RNA synthetases through their accurate aminoacylation of cognate tRNAs and their ability to correct occasional errors of aminoacylation by editing reactions[1–5]. A principal source of mistranslation comes from mistaking glycine or serine for alanine, which can lead to serious cell and animal pathologies, including neurodegeneration[3]. A single specific G•U base pair (G3•U70) marks a tRNA for aminoacylation by alanyl-tRNA synthetase[6–9]. Mistranslation occurs when glycine or serine is joined to the G3•U70-containing tRNAs, and is prevented by the editing activity that clears the mischarged amino acid. Previously it was assumed that the specificity for recognition of $tRNA^{Ala}$ for editing was provided by the same structural determinants as used for aminoacylation. Here we show that the editing site of alanyl-tRNA synthetase, as an artificial recombinant fragment, targets mischarged $tRNA^{Ala}$ using a structural motif unrelated to that for aminoacylation so that, remarkably, two motifs (one for aminoacylation and one for editing) in the same enzyme independently can provide determinants for $tRNA^{Ala}$ recognition. The structural motif for editing is also found naturally in genome-encoded protein fragments that are widely distributed in evolution[10–12]. These also recognize mischarged $tRNA^{Ala}$. Thus, through evolution, three different complexes with the same tRNA can guard against mistaking glycine or serine for alanine.

Mistranslation results from insertion of amino acids at wrong codons[1,2,5]. Aminoacyl-tRNA synthetases (aaRSs) provide the main mechanism for production of mischarged tRNAs. These enzymes catalyse attachment of amino acids in a two-step reaction:

$$aa + ATP + aaRS \rightarrow aaRS(aa\text{–}AMP) + PP_i \quad (1)$$

$$aaRS(aa\text{–}AMP) + tRNA \rightarrow aa\text{–}tRNA + AMP + aaRS \quad (2)$$

where the amino acid (aa) is condensed with ATP to form a tightly bound aminoacyl adenylate and PP_i is released (equation (1)). The activated aminoacyl group is then transferred from the adenylate to the 3′-end of the tRNA to form aa–tRNA with liberation of AMP and regeneration of enzyme (equation (2)). However, some aaRSs make errors during the amino acid activation step owing to the inherent physiochemical limitations on closely discriminating similar amino acid side chains[13]. An example is alanyl-tRNA synthetase (AlaRS), which misactivates Gly and Ser to yield $Gly\text{–}tRNA^{Ala}$ and $Ser\text{–}tRNA^{Ala}$, respectively[1,14]. These errors are cleared by a second active site, which is specifically designed for hydrolytic editing:

$$Ser\text{–}tRNA^{Ala} \rightarrow Ser + tRNA^{Ala} \text{ or } Gly\text{–}tRNA^{Ala} \rightarrow Gly + tRNA^{Ala} \quad (3)$$

Thus, this special activity prevents mistranslation.

Figure 1a schematically illustrates the design of *Escherichia coli* AlaRS (encoded by *alaS*)[15], a polypeptide consisting of 875 amino acids. The amino-terminal 461 amino acids encode the catalytic

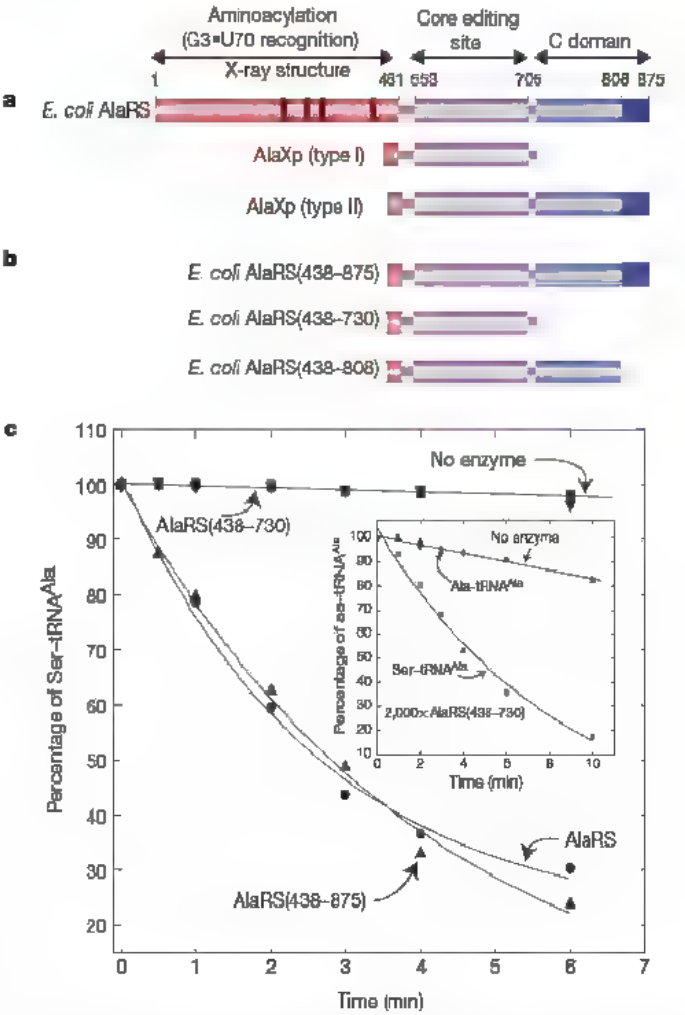


Figure 1 | Domains of AlaRS and AlaXp and deacylation activity of selected fragments. a, Domains of *E. coli* AlaRS and type I and II AlaXps. The domain for amino acid activation and G3•U70 $tRNA^{Ala}$ recognition is red (vertical bars show determinants for acceptor stem and G3•U70 recognition). The editing domain is violet and the C domain is blue (with the highly conserved portion in dark blue). **b**, Constructs used in this study. **c**, Deacylation of $Ser\text{–}tRNA^{Ala}$ by wild-type AlaRS (circles), *E. coli* AlaRS(438–875) (upright triangles), *E. coli* AlaRS(438–730) (squares) or a no-enzyme control (inverted triangles). Inset, specific deacylation of $Ser\text{–}tRNA^{Ala}$ (squares) and $Ala\text{–}tRNA^{Ala}$ (triangles) by 2,000× the concentration of AlaRS(438–730), or a no-enzyme control (circles). Data shown represent a typical experiment.

[1]Department of Molecular Biology and Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037 USA.
*These authors contributed equally to this work.

domain for aminoacylation, which is strictly dependent on a G3•U70 base pair in the acceptor stem of $tRNA^{Ala}$ for aminoacylation[6–9]. The G3•U70-dependent recognition of $tRNA^{Ala}$ is conserved from bacteria to humans and is so robust that transfer of G3•U70 into a non-alanine tRNA can be sufficient to confer aminoacylation with alanine. The three-dimensional structure of the aminoacylation domain of *Aquifex aeolicus* AlaRS[9] corresponds essentially to the N-terminal 461-amino-acid fragment of *E. coli* AlaRS (AlaRS(1–461)). This fragment is sufficient for aminoacylation *in vitro* and *in vivo*[16], and the determinants (marked in Fig. 1a) for specific (G3•U70-dependent) recognition of $tRNA^{Ala}$ include Asp 235, Asp 285, Arg 314 and Ala 409 and are embedded in the structural format of a class II tRNA synthetase with its seven-stranded β-structure and flanking α-helices[9,17,18].

For class II tRNA synthetases such as ThrRS (encoded by thrS) and AlaRS, a special insertion or fusion provides the centre for editing[1,19,20]. For *E. coli* AlaRS, this editing centre is encoded by the region from 553–705 (Fig. 1a). Mild editing defects, arising from mutations in the germ line, can be vertically transmitted in the population. Indeed, a minor defect (twofold) in the hydrolytic editing activity of AlaRS leads to heritable ataxia in the mouse[3]. This ataxia is caused by neuronal degeneration of Purkinje cells in the cerebellum, associated with mistranslation-induced triggering of the unfolded protein response. The extreme sensitivity of cells (from bacteria[1,2,4,21,22] to mammals[3,5]) to editing defects provides a straightforward rationale for why the editing domain of AlaRS is conserved through evolution. This domain is believed to have been present in the last common ancestor of the tree of life that split into the three great domains: Archaea, Bacteria and Eukarya[1,23].

Genome-encoded, active free-standing fragments homologous to editing domains of tRNA synthetases, including AlaRS, are widely distributed in nature[10–12]. The domains that most closely resemble the editing domain of AlaRS are referred to as AlaXps, and we classify them into two different types on the basis of sequence (Fig. 1a). Type I AlaXps have the core editing domain and a modest amount of flanking sequence. Type II AlaXps have all of the sequence of type I AlaXps, but in addition have an extended carboxy domain much like that found in AlaRSs. Because in our preliminary work several type II AlaXps were active for editing in catalytic amounts, we considered the possibility that the active site for aminoacylation was not needed for capture of mischarged tRNA. Instead, we reasoned that simple fusion of the type II AlaXp-like piece was a straightforward way to provide an editing function for AlaRS. Not clear was how either the type I or the type II AlaXps recognized mischarged $tRNA^{Ala}$ and, furthermore, whether that recognition mechanism was also used by the editing site of AlaRS.

To separate out the functional components of AlaRS required for editing and tRNA recognition, deletion mutants lacking the catalytic site for aminoacylation were created. The design of these mutants was roughly based on the sequences of AlaXps, either type I (*E. coli* AlaRS(438–730)) or type II (*E. coli* AlaRS(438–875)) (see Fig. 1b). Because the natural AlaXp fragments deacylate misacylated $tRNA^{Ala}$, deletions based on their sequences provided a logical framework for construction of deletions in AlaRS.

Both *E. coli* AlaRS(438–730) (homologous to type I AlaXp) and *E. coli* AlaRS(438–875) (homologous to type II AlaXp) were tested for their ability to deacylate Ser–$tRNA^{Ala}$. Whereas *E. coli* AlaRS (438–875) was fully active for clearance of Ser–$tRNA^{Ala}$, *E. coli* AlaRS(438–730) was inactive (Fig. 1c). Using the nitrocellulose filter RNA-binding assay[24], we demonstrated that the inactivity of *E. coli* AlaRS(438–730) correlated with a lack of binding of $tRNA^{Ala}$ (Supplementary Fig. 1). However, at much higher concentrations, *E. coli* AlaRS(438–730) was capable of specifically deacylating misacylated $tRNA^{Ala}$ (Fig. 1c, inset). Thus, the catalytic site for editing was not disrupted in *E. coli* AlaRS(438–730); instead, the reduction in editing activity resulted from a loss of affinity for tRNA.

Still unclear was whether fragment *E. coli* AlaRS(438–875), which lacks the G•U-specific determinants of the aminoacylation domain, would be specific for $tRNA^{Ala}$. Although *E. coli* AlaRS(438–875) cleared mischarged $tRNA^{Ala}$ with robust activity, it failed to deacylate Ser–$tRNA^{Thr}$ (Fig. 2a), mirroring the specificity observed with isolated type I AlaXp and full-length AlaRS[10]. Because *E. coli* AlaRS(438–875) deacylated diverse chimaeric tRNAs that had the acceptor stem of $tRNA^{Ala}$ but in which the rest of the sequence was swapped for a different tRNA sequence (Supplementary Fig. 2a, b), the specificity of *E. coli* AlaRS(438–875) must be governed by the acceptor stem of $tRNA^{Ala}$. As shown in Fig. 1c (inset), high concentrations of *E. coli* AlaRS(438–730) deacylated Ser–$tRNA^{Ala}$; however, the same or higher concentrations of this fragment failed to deacylate Ser–$tRNA^{Thr}$ (Fig. 2a).

Thus, specific determinants for the recognition of $tRNA^{Ala}$ are embedded in *E. coli* AlaRS(438–730). These determinants are inherent to the core editing domain and are, presumably, shared with both type I and II AlaXps, which deacylate misacylated tRNAs with high efficiency[10,25,26] (K.B. and P.S., unpublished). Separate from these specificity determinants are extra C-terminal non-specific RNA-binding elements between amino acids 730 and 875 of

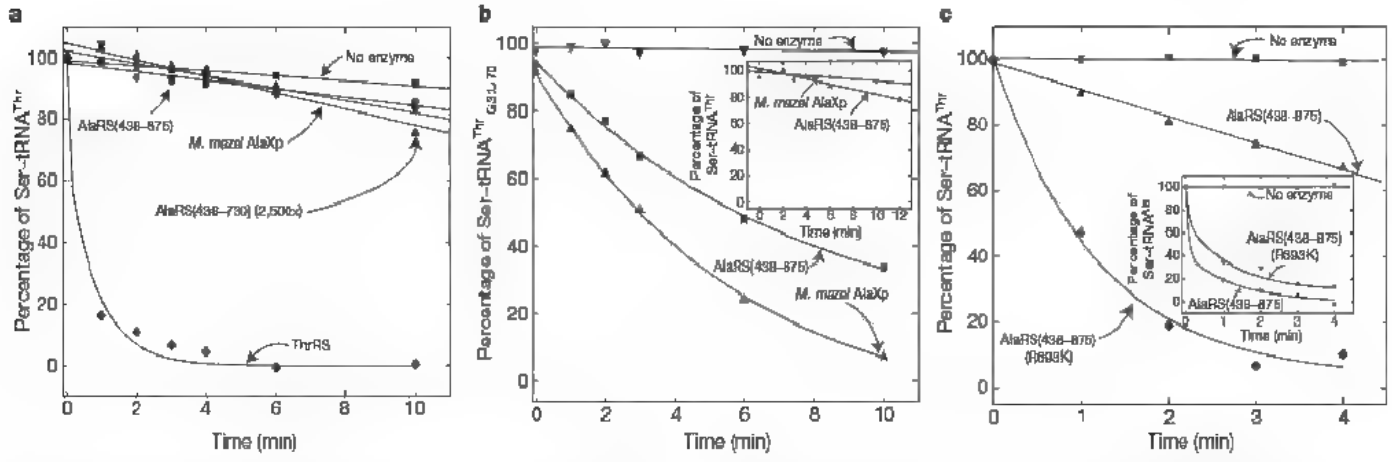


Figure 2 | AlaRS and its fragments are specific for $tRNA^{Ala}$ and influenced by the G3•U70 base pair. **a**, Deacylation of Ser–$tRNA^{Thr}$ with ThrRS (diamonds), *E. coli* AlaRS(438–875) (circles), *M. mazei* AlaXp (a type I AlaXp) (inverted triangles), 2,500× concentration of *E. coli* AlaRS(438–730) (upright triangles) or a no-enzyme control (squares). **b**, Installing the G3•U70 base pair into $tRNA^{Thr}$ ($tRNA^{Thr}_{G3U70}$) triggers recognition by *E. coli* AlaRS(438–875) (squares) and type I *M. mazei* AlaXp (triangles). A no-enzyme control is shown above (inverted triangles). Inset, control showing lack of deacylation of Ser–$tRNA^{Thr}$ by *E. coli* AlaRS(438–875) (triangles) and type I *M. mazei* AlaXp (circles). **c**, Perturbation of a conserved 'RR' motif leads to loss in G3•U70 selectivity. Deacylation activity of *E. coli* AlaRS(438–875) (triangles), *E. coli* R693K AlaRS(438–875) (diamonds), or a no-enzyme control (squares) towards Ser–$tRNA^{Thr}$. Inset, deacylation of Ser–$tRNA^{Ala}$ by *E. coli* AlaRS(438–875) (triangles), *E. coli* R693K AlaRS(438–875) (inverted triangles) or a no-enzyme control (squares). Data shown represent a typical experiment.

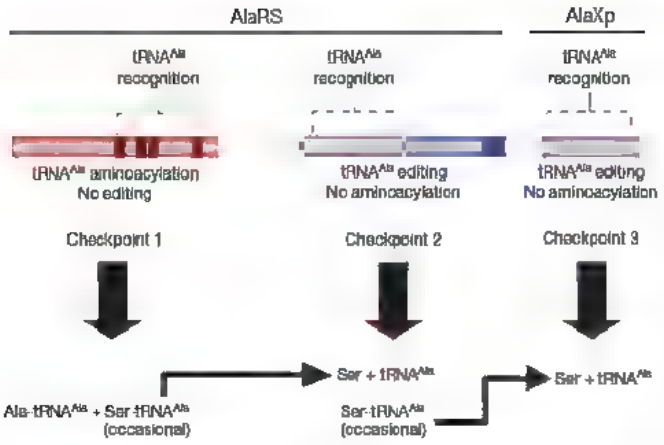


Figure 3 | Multiple checkpoints of $tRNA^{Ala}$ recognition for prevention of mistranslation. The first checkpoint occurs in the N-terminal domain in recognition of $tRNA^{Ala}$ and discrimination of alanine versus serine (or glycine)[16]. The result is the occasional production of Ser–$tRNA^{Ala}$ (refs 1, 3, 14). This minor product is then recognized in a $tRNA^{Ala}$-dependent manner and cleared by a second checkpoint (the editing domain). Finally, any residual Ser–$tRNA^{Ala}$ that remains can be cleared by a third checkpoint (AlaXp) that also recognizes $tRNA^{Ala}$. The net effect is that the quantity of misacylated $tRNA^{Ala}$ is reduced, and mistranslation from confusing serine or glycine for alanine is prevented.

AlaRS(438–875) (Fig. 1c and Supplementary Fig. 1). To compensate for lacking the extra RNA-binding determinants of the C domain of AlaRSs and type II AlaXps, type I AlaXps have adapted the core editing domain with basic residues to interact more strongly with $tRNA^{Ala}$ (refs 25, 26 and Supplementary Fig. 3a, b). In the case of the C domain of AlaRSs and type II AlaXps, we showed in separate experiments that the needed tRNA interaction energy is further localized to non-specific RNA-binding determinants located in the region between amino acids 808 and 875 (Supplementary Fig. 4).

To investigate further the specificity of the newly found determinants for tRNA recognition in the region outside the domain for aminoacylation, we installed the G3•U70 base pair into Ser–$tRNA^{Thr}$ to produce Ser–$tRNA^{Thr}_{G3•U70}$. Even though *E. coli* AlaRS(438–875) and the *Methanosarcina mazeii* type I AlaXp lack the aminoacylation domain of AlaRS, both deacylated Ser–$tRNA^{Thr}_{G3•U70}$ suggesting that they recognize tRNA for deacylation based, at least in part, on a G3•U70 base pair (Fig. 2b). At the same concentration of enzyme used to deacylate Ser–$tRNA^{Thr}_{G3U70}$, these enzymes failed to significantly deacylate Ser–$tRNA^{Thr}$ (Fig. 2b, inset). A region important for tRNA-specificity was further localized to a predicted strand–loop–strand motif within *E. coli* AlaRS(438–875). In particular, Arg693 in the strand–loop–strand motif is highly conserved between AlaRSs and AlaXps and, on the basis of existing structural information, can be modelled to be close to the 3•70 base pair of $tRNA^{Ala}$ (Supplementary Fig. 5)[26]. Notably, mutant R693K *E. coli* AlaRS(438–875) had relaxed specificity for $tRNA^{Thr}$, and deacylated Ser–$tRNA^{Thr}$ (Fig. 2c). Thus, the *E. coli* AlaRS editing domain and *M. mazei* type I AlaXp share a second, independent way to recognize $tRNA^{Ala}$.

This work shows that AlaRS contains two protein motifs for specific recognition of $tRNA^{Ala}$: one well-studied set of amino acids in the aminoacylation domain[10,26] and a second, unrelated, structural motif within the editing domain between residues 680 and 699 (Fig. 3). Notably, the data in Figs 1c and 2 show that each of these motifs can recognize $tRNA^{Ala}$ in the absence of the other. The need for two distinct motifs for recognition of $tRNA^{Ala}$ in the same tRNA synthetase can be rationalized from the severe neurodegeneration in the mouse resulting from even a mild level of mistranslation, in which both glycine and serine were confused for alanine[3]. In addition to the two $tRNA^{Ala}$ recognition elements imbedded within AlaRS (Fig. 3), in many organisms (including the mouse) a third mechanism for capture of $tRNA^{Ala}$ is provided by genome-encoded AlaXp fragments (Fig. 3). Even though type I AlaXps lack the N-terminal-specific tRNA-binding elements of AlaRSs, we showed here an example from *M. mazei* that specifically recognized $tRNA^{Ala}$. Mouse AlaXp (also known as Aarsd1) also deacylated misacylated $tRNA^{Ala}$ (Supplementary Fig. 6; K.B. and P.S, unpublished). However, we could detect no complex (by pull-down assays) between mouse AlaXp and mouse AlaRS (Aars) (as reported for *E. coli* ProRS, and the *Haemophilus influenzae* free-standing editing fragment YbaK[12]), consistent with AlaXp working in isolation in the mouse. It is of interest to determine whether multiple checkpoints guarding against mistranslation are operative through other tRNAs, such as the occasional confusion of serine for threonine that comes from mischarging of $tRNA^{Thr}$ by ThrRS, which has an AlaRS-like editing domain for clearing serine from $tRNA^{Thr}$.

METHODS SUMMARY

Preparation of materials. Constructs described were prepared by PCR of the targeted sequence and cloning of the PCR product. Recombinant protein was produced by *E. coli* overexpression and Ni-NTA purification. The concentration of purified proteins was determined by Bradford assay. Transfer RNA was produced by either *in vivo* overexpression[27] or *in vitro* transcription[28]. Correctly acylated tRNAs were produced by extraction and size exclusion purification of a mixture containing tritiated cognate amino acid, aaRS and the purified tRNA. An aaRS bearing a mutation in the editing site was used for producing incorrectly acylated tRNA. The quantity of acylated tRNA was determined by A_{260}.

Deacylation assays. Assays were performed at 25 °C (pH 7.5) with assay buffer (50 mM HEPES (pH 7.5), 20 mM KCl, 2 mM DTT and 10 mM $MgCl_2$) in 96-well plates as described[29]. Enzyme dilution buffer was added instead of enzyme to determine background hydrolysis. To determine 100% product (and therefore percentage of aa–tRNA remaining), NaOH (20–50 mM) was added to the reaction to liberate all aa from the tRNA during the time course.

The enzyme concentration in Fig. 1c was 10 nM (inset, 20 μM). For Fig. 2a, 10 nM was used for all enzymes except *E. coli* AlaRS(438–730) (25 μM). The assay in Fig. 2b used 200 nM (inset, same concentration). Finally, for the assay in Fig. 2c, 625 nM was used (inset, same concentration). Each enzyme activity towards each substrate was independently verified a minimum of three times (the exception being the ThrRS positive control in Fig. 2a).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 28 August; accepted 7 November 2007.

1. Beebe, K., Ribas De Pouplana, L. & Schimmel, P. Elucidation of tRNA-dependent editing by a class II tRNA synthetase and significance for cell viability. *EMBO J.* 22, 668–675 (2003).
2. Doring, V. *et al.* Enlarging the amino acid set of *Escherichia coli* by infiltration of the valine coding pathway. *Science* 292, 501–504 (2001).
3. Lee, J. W. *et al.* Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature* 443, 50–55 (2006).
4. Nangle, L. A., De Crecy Lagard, V., Doring, V. & Schimmel, P. Genetic code ambiguity. Cell viability related to the severity of editing defects in mutant tRNA synthetases. *J. Biol. Chem.* 277, 45729–45733 (2002).
5. Nangle, L. A., Motta, C. M. & Schimmel, P. Global effects of mistranslation from an editing defect in mammalian cells. *Chem. Biol.* 13, 1091–1100 (2006).
6. Hou, Y. M. & Schimmel, P. A simple structural feature is a major determinant of the identity of a transfer RNA. *Nature* 333, 140–145 (1988).
7. Hou, Y. M. & Schimmel, P. Evidence that a major determ nant for the identity of a transfer RNA is conserved in evolution. *Biochemistry* 28, 6800–6804 (1989).
8. McClain, W. H. & Foss, K. Changing the acceptor identity of a transfer RNA by altering nucleotides in a "variable pocket". *Science* 241, 1804–1807 (1988).
9. Swairjo, M. A. *et al.* Alanyl-tRNA synthetase crystal structure and design for acceptor-stem recognition. *Mol. Cell* 13, 829–841 (2004).
10. Ahel, I., Korencic, D., Ibba, M. & Soll, D. Trans-editing of mischarged tRNAs. *Proc. Natl Acad. Sci. USA* 100, 15422–15427 (2003).
11. An, S. & Musier-Forsyth, K. Trans-editing of Cys-tRNAPro by *Haemophilus influenzae* YbaK protein. *J. Biol. Chem.* 279, 42359–42362 (2004).
12. An, S. & Musier-Forsyth, K. Cys-$tRNA^{Pro}$ editing by *Haemophilus influenzae* YbaK via a novel synthetase•YbaK tRNA ternary complex. *J. Biol. Chem.* 280, 34465–34472 (2005).
13. Pauling, L. in *Festschrift fur Prof. Dr. Arthur Stoll* 597–602 (Birkhauser Basel, 1957).
14. Tsui, W. C. & Fersht, A. R. Probing the principles of amino acid selection using the alanyl-tRNA synthetase from *Escherichia coli*. *Nucleic Acids Res.* 9, 4627–4637 (1981).

15. Putney, S. D. *et al.* Primary structure of a large aminoacyl-tRNA synthetase. *Science* 213, 1497–1501 (1981).
16. Jasin, M., Regan, L. & Schimmel, P. Modular arrangement of functional domains along the sequence of an aminoacyl tRNA synthetase. *Nature* 306, 441–447 (1983).
17. Cusack, S. *et al.* A second class of synthetase structure revealed by X-ray analysis of *Escherichia coli* seryl-tRNA synthetase at 2.5 Å. *Nature* 347, 249–255 (1990).
18. Ruff, M. *et al.* Class II aminoacyl transfer RNA synthetases: crystal structure of yeast aspartyl-tRNA synthetase complexed with tRNA(Asp). *Science* 252, 1682–1689 (1991).
19. Dock-Bregeon, A. *et al.* Transfer RNA-mediated editing in threonyl-tRNA synthetase. The class II solution to the double discrimination problem. *Cell* 103, 877–884 (2000).
20. Sankaranarayanan, R. *et al.* Zinc ion mediated amino acid discrimination by threonyl-tRNA synthetase. *Nature Struct. Biol.* 7, 461–465 (2000).
21. Karkhanis, V. A., Boniecki, M. T., Poruri, K. & Martinis, S. A. A viable amino acid editing activity in the leucyl-tRNA synthetase CP1-splicing domain is not required in the yeast mitochondria. *J. Biol. Chem.* 281, 33217–33225 (2006).
22. Roy, H., Ling, J., Irnov, M. & Ibba, M. Post-transfer editing *in vitro* and *in vivo* by the β subunit of phenylalanyl-tRNA synthetase. *EMBO J.* 23, 4639–4648 (2004).
23. Schimmel, P. & Ribas de Pouplana, L. Formation of two classes of tRNA synthetases in relation to editing functions and genetic code. *Cold Spring Harb. Symp. Quant. Biol.* 66, 161–166 (2001).
24. Regan, L., Bowie, J. & Schimmel, P. Polypeptide sequences essential for RNA recognition by an enzyme. *Science* 235, 1651–1653 (1987).
25. Fukunaga, R. & Yokoyama, S. Structure of the AlaX-M trans-editing enzyme from *Pyrococcus horikoshii*. *Acta Crystallogr. D* 63, 390–400 (2007).
26. Sokabe, M. *et al.* Molecular basis of alanine discrimination in editing site. *Proc. Natl Acad. Sci. USA* 102, 11669–11674 (2005).
27. Waas, W. F. & Schimmel, P. Evidence that tRNA synthetase-directed proton transfer stops mistranslation. *Biochemistry* 46, 12062–12070 (2007).
28. Beebe, K., Merriman, E. & Schimmel, P. Structure-specific tRNA determinants for editing a mischarged amino acid. *J. Biol. Chem.* 278, 45056–45061 (2003).
29. Beebe, K. *et al.* A universal plate format for increased throughput of assays that monitor multiple aminoacyl transfer RNA synthetase activities. *Anal. Biochem.* 368, 111–121 (2007).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank P. O'Maille for his gift of plasmid pH8GW and M. Sokabe for discussions about the model of the tRNA complex with *Pyrococcus horikoshi* AlaXp. This work was supported by grants from the National Institutes of Health, the Skaggs Foundation, and the National Foundation for Cancer Research.

Author Contributions K.B., M.M. and E.M. performed experiments and produced all materials. K.B., M.M. and P.S. conceived ideas, designed experiments, and wrote and edited the manuscript. All authors reviewed and approved the final manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to P.S. (schimmel@scripps.edu).

LETTERS

Structure of a tyrosyl-tRNA synthetase splicing factor bound to a group I intron RNA

Paul J. Paukstelis[1], Jui-Hui Chen[2], Elaine Chase[2], Alan M. Lambowitz[1]* & Barbara L. Golden[2]*

The 'RNA world' hypothesis holds that during evolution the structural and enzymatic functions initially served by RNA were assumed by proteins, leading to the latter's domination of biological catalysis. This progression can still be seen in modern biology, where ribozymes, such as the ribosome and RNase P, have evolved into protein-dependent RNA catalysts ('RNPzymes'). Similarly, group I introns use RNA-catalysed splicing reactions, but many function as RNPzymes bound to proteins that stabilize their catalytically active RNA structure[1,2]. One such protein, the *Neurospora crassa* mitochondrial tyrosyl-tRNA synthetase (TyrRS; CYT-18), is bifunctional and both aminoacylates mitochondrial $tRNA^{Tyr}$ and promotes the splicing of mitochondrial group I introns[3]. Here we determine a 4.5-Å co-crystal structure of the Twort *orf*142-I2 group I intron ribozyme bound to splicing-active, carboxy-terminally truncated CYT-18. The structure shows that the group I intron binds across the two subunits of the homodimeric protein with a newly evolved RNA-binding surface distinct from that which binds $tRNA^{Tyr}$. This RNA binding surface provides an extended scaffold for the phosphodiester backbone of the conserved catalytic core of the intron RNA, allowing the protein to promote the splicing of a wide variety of group I introns. The group I intron-binding surface includes three small insertions and additional structural adaptations relative to non-splicing bacterial TyrRSs, indicating a multistep adaptation for splicing function. The co-crystal structure provides insight into how CYT-18 promotes group I intron splicing, how it evolved to have this function, and how proteins could have incrementally replaced RNA structures during the transition from an RNA world to an RNP world.

The group I intron catalytic core has a conserved three-dimensional structure consisting of two extended RNA domains, P4–P6 and P3–P9, which interact to form the intron's active site (Fig. 1a, b)[4–7]. This active site aligns the splice sites and guanosine substrate and uses specifically bound Mg^{2+} ions to catalyse splicing by means of guanosine-initiated transesterification reactions. CYT-18 recognizes highly conserved secondary and tertiary structural features of the catalytic core of the intron RNA and is unique among group I intron splicing factors in being able to promote the splicing of various group I introns provided that the catalytic core is accessible[8–11]. CYT-18 and homologous bacterial TyrRSs consist of a nucleotide-binding-fold domain followed by α-helical and C-terminal domains, and they function as homodimers, with the nucleotide-binding fold of one subunit binding the acceptor stem of the tRNA, and the α-helical and C-terminal domains of the other subunit binding the anticodon and variable arms of the tRNA[3,12,13]. However, only the *N. crassa* mitochondrial TyrRS and that of the closely related fungus *Podospora anserma* have been found to function in group I intron splicing, suggesting that adaptation of the conserved structure is required for splicing activity[12,14].

We recently determined a crystal structure of CYT-18/Δ424–669, which lacks the flexibly attached C-terminal domain but still promotes the splicing of most group I introns[12]. Models based on this structure combined with biochemical data suggested that the protein does not recognize tRNA-like features of the intron as such, but uses instead a distinct RNA-binding surface that includes an α-helical amino-terminal extension (H0) and two other small insertions (Ins1 and Ins2), which are absent from non-splicing bacterial TyrRSs[12].

Here we determine by molecular replacement a co-crystal structure of CYT-18/Δ424–669 bound to the bacteriophage Twort *orf*142-I2 group I ribozyme, using data extending to 4.5 Å resolution. At this resolution, interacting regions of the protein and RNA are clearly discernible, but local conformational changes could go undetected (Fig. 1a and Supplementary Fig. 1). The asymmetric unit is composed of four nearly identical complexes related by non-crystallographic symmetry, which significantly improves the parameter-to-observation ratio (Supplementary Table 1). Except for RNA regions

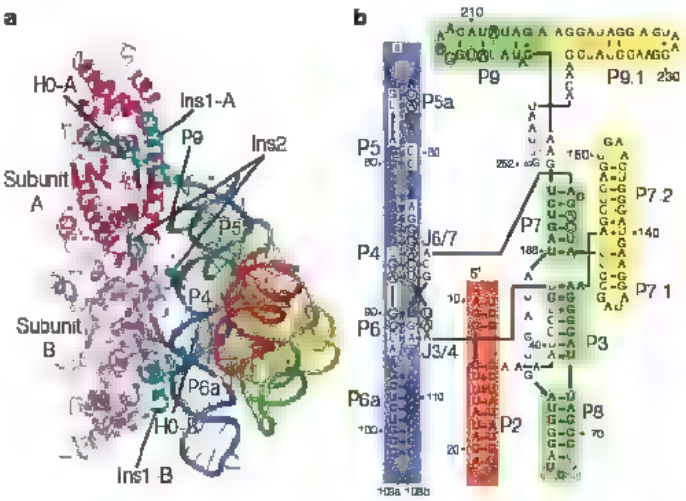


Figure 1 | Co-crystal structure of the Twort *orf*142-I2 group I intron ribozyme bound to CYT-18/Δ424–669. a, Ribbon diagram. CYT-18 subunits A and B are coloured magenta and violet, respectively, and CYT-18-specific insertions H0, Ins1 and Ins2 are coloured cyan. **b**, Secondary structure of the Twort *orf*142-I2 intron ribozyme showing nucleotide residues within 4 Å of the protein in the co-crystal structure (circled). Boxed nucleotide residues correspond to phosphodiester-backbone positions protected by full-length CYT-18 in the *N. crassa ND1* intron[11,18]. Protections in P3, P5 and P8, which are not seen in the co-crystal structure, are attributable to the C-terminal domain of CYT-18, which is absent from CYT-18/Δ424–669 (see Fig. 3)[12].

[1]Institute for Cellular and Molecular Biology, Department of Chemistry and Biochemistry, and Section of Molecular Genetics and Microbiology, School of Biological Sciences, University of Texas at Austin, Austin, Texas 78712, USA. [2]Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA.
*These authors contributed equally to this work

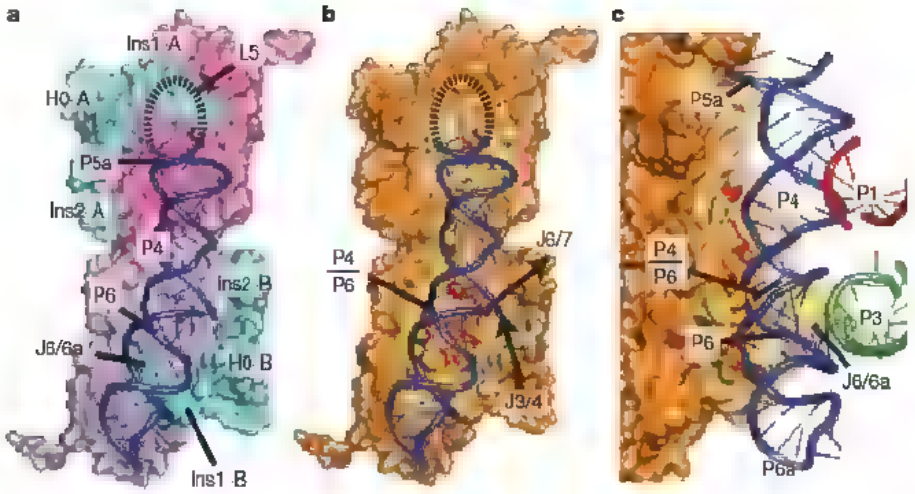


Figure 2 | CYT-18 binding to the P4–P6 domain. **a**, CYT-18 binds the length of the P4–P6 domain, with the CYT-18-specific insertions (cyan) contributing to the formation of binding pockets for different regions. CYT-18 is drawn as a surface model, with subunits A and B coloured magenta and violet, respectively. **b**, **c**, Putative contacts between CYT-18 and the P4–P6 domain. The surfaces of protein atoms within 4 Å of the RNA are coloured red, atoms within 4–5 Å yellow, atoms within 5–10 Å peach, and atoms farther away than 10 Å orange. The views in **b** and **c** are rotated by 90° around the vertical axis.

involved in crystal contacts or not previously visible, the protein and RNA structures in the complex deviate little from those for the unbound molecules (root mean squared deviations 0.909 and 0.996 Å for Cα atoms of the two protein subunits, and 1.56 Å for the RNA; Supplementary Fig. 2). Because the individual RNA and protein structures were determined at significantly higher resolution[7,12] and seem largely unchanged on binding, the data yield a pseudo-atomic model of the complex. Functional binding of CYT-18/Δ424–669 to the Twort ribozyme is indicated by a 17-fold increased k_{cat} for RNA substrate cleavage under single-turnover conditions in reaction medium containing a low (1 mM) Mg^{2+} concentration (from 0.015 to 0.26 min^{-1}; not shown). Further, the crystal structure is supported by large amounts of biochemical data identifying RNA–protein interaction sites (Supplementary Table 3), including distance restraints determined by site-directed hydroxyl radical cleavage of the RNA from 11 different amino-acid positions[12] (Supplementary Fig. 3).

The structure shows that the Twort RNA binds across the two CYT-18 subunits (denoted A and B), with the protein contacting both the P4–P6 and P3–P9 domains of the catalytic core of the intron but not peripheral RNA structures. The intron RNA-binding surface is electropositive[12] but does not overlap that which binds $tRNA^{Tyr}$ (Supplementary Fig. 4). Overall, the RNA–protein interface excludes 1,721 $Å^2$ of solvent-accessible surface area, with most being due to subunit B interactions (1,568 $Å^2$)[15].

The P4–P6 domain of group I introns is a rod-like structure formed by the stacking of the P4 and P6 helices (Fig. 2). Previous biochemical studies indicated that CYT-18 interacts extensively with the P4–P6 domain and promotes its assembly in part by helping to establish the correct geometry around the P4–P6 helical junction[16,17]. The structure shows that CYT-18 binds along one face of the coaxially stacked P4–P6 helices, with the insertions unique to splicing-competent mitochondrial TyrRSs helping to create RNA-binding pockets (Fig. 2 and Supplementary Fig. 5). The protein binds to the P4–P6 domain as follows: first, in the minor groove at the P4–P6 junction; second, below the P4–P6 junction with Ins1-B jutting into the major groove of P6 and P6a; and third, above the P4–P6 junction in the minor groove of P5a. Notably, the P4–P6 junction binds in a pocket formed from β-strand D and helix 5 of subunit B, and P5a binds in the same pocket of subunit A (Fig. 2 and Supplementary Fig. 5a). Collectively, these interactions form a clamp that may stabilize the correct conformation of the P4–P6 stacked helices.

In addition to contacting the P4–P6 domain, biochemical studies indicated that CYT-18 also contacts the P3–P9 domain to stabilize the two domains in the correct relative orientation to form the intron's active site[11,18,19]. The relative orientation of the P4–P6 and P3–P9 domains in group I introns is determined principally by four tertiary interactions: the J3/4 and J6/7 nucleotide triples in the minor groove of P6 and major groove of P4, respectively; docking of P3 in the minor groove of J6/6a; and the L9–P5 tetraloop-receptor interaction[5–7]. Strikingly, the structure shows that CYT-18 binds in a position to promote and/or stabilize all of these interactions, again with key roles for the CYT-18-specific insertions (Fig. 3).

The J3/4 junction region is seen in the structure to interact with the N-terminal extension H0-B and Ins1-B of CYT-18 (Fig. 3). Tyr 41-B is found between U48 and U49, potentially inducing a kink that forces J3/4 into the P6 minor groove for triple formation (Supplementary Fig. 6a), in agreement with previous findings that CYT-18 promotes the J3/4 nucleotide triple interaction[20,21].

In J6/7, the first two nucleotides (G117 and C118) form major-groove triples with the first two base pairs of P4, and the last

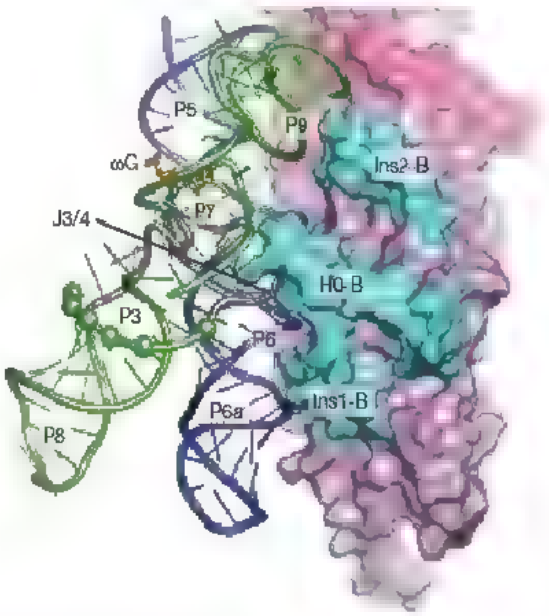


Figure 3 | CYT-18-specific insertions bridge the P4–P6 and P3–P9 domains. RNA and protein are coloured as in Fig. 1. The nucleophilic guanosine residue (ωG) bound at P7 is shown in orange. Spheres show backbone phosphates in P3 that are putatively protected by the C-terminal domain of CYT-18, which is absent from CYT-18/Δ424–669 (see Fig. 1).

nucleotide (A119) interacts with P7 to help in forming the guanosine-binding site[7]. The CYT-18 core interacts with the same P4 base pairs in the minor groove (see above) and H0-B interacts directly with P7. Thus, the protein potentially stabilizes both the J6/7 interactions and the guanosine-binding site (Fig. 3 and Supplementary Fig. 6a).

The P3–J6/6a interaction may be promoted by CYT-18 in two ways. First, the previously described docking of Ins1-B in the major groove of P6 and P6a (Fig. 2a) may help to establish the correct conformation of J6/6a for the docking of P3 in the minor groove. Second, biochemical evidence suggests that the C-terminal domain of CYT-18, which is absent from CYT-18/Δ424–669, binds P3 (Figs 1b and 3)[11,12].

Finally, the interaction between the L9 tetraloop and the minor groove of P5 seems to be stabilized by direct contacts with the protein. The L9 tetraloop is positioned by the binding of Ins2-B within the minor groove of P9 and by contacts to H5-A, whereas P5 is oriented by the previously described interaction between subunit A and P5a (Fig. 3 and Supplementary Fig. 6b).

The *N. crassa* mitochondrial group I introns that are natural substrates for CYT-18 form by themselves most of the conserved secondary structure, but they form little tertiary structure even at high Mg^{2+} concentrations[18]. The crystal structure is consistent with previous biochemical and genetic analyses, which suggested that CYT-18 binds first to the P4–P6 domain to promote its assembly and then makes additional contacts with the P3–P9 domain to stabilize the correct relative orientation of the two domains to form the active site of the intron RNA[11,18,19]. The latter step could occur by CYT-18 serving as a scaffold for tertiary structure nucleation, tertiary structure capture, or some combination of these mechanisms[18,22].

The structure suggests that CYT-18 interacts almost exclusively with the intron's phosphodiester backbone, with the only potential base contacts between residues in Ins2-B and P9, and between H0-B and J3/4. These findings agree with previous chemical footprinting experiments, which showed similarly positioned phosphate-backbone protections in the *N. crassa ND1*, mitochondrial large subunit rRNA (LSU) and yeast bI5 introns, and few if any base contacts (Fig. 1b shows the correspondence of phosphate-backbone contacts for the *ND1* intron)[11,18,19]. Recognition of the three-dimensional structure of the phosphodiester backbone enables CYT-18 to bind various group I introns, which have little primary sequence similarity but highly conserved secondary and tertiary structures[5–7].

The co-crystal structure provides a striking snapshot of how structural functions of RNA can be assumed by proteins. Previous work showed that CYT-18 could replace the peripheral RNA structure P5abc to promote the splicing at low Mg^{2+} concentrations of a *Tetrahymena thermophila* LSU intron derivative lacking this structure[10]. Both CYT-18 and P5abc bind the length of the P4–P6 domain with contacts at P5, the P4–P6 junction and a distal region of P6 (P6a for CYT-18 and J6a/6b for P5abc), enabling them to stabilize the backbone conformation on both sides of the P4–P6 junction (Fig. 4). However, P5abc is sequence specific, stabilizing the P4–P6 junction by means of A-minor contacts in the minor groove[23], whereas CYT-18 seems to contact only the phosphodiester backbone in this region. CYT-18 also differs from P5abc in contacting the P3–P9 domain in addition to the P4–P6 domain. These further interactions explain the ability of CYT-18 to compensate not only for mutations in the P4–P6 domain but also for those that weaken tertiary interactions between the P4–P6 and P3–P9 domains[16,20,24].

The structure also shows how the unique structural adaptations of the *N. crassa* mitochondrial TyrRS are related to splicing activity. Thus, the CYT-18-specific insertions H0, Ins1 and Ins2, which had been implicated previously in splicing activity[12,25], are all seen to interact directly with the intron RNA and potentially to stabilize key tertiary interactions. Further, the protein core has additional structural adaptations, including basic amino-acid substitutions relative to non-splicing bacterial TyrRSs that contribute to group I intron binding (Supplementary Table 3)[12].

Finally, analysis of genome sequences showed that H0, Ins1 and Ins2 are uniquely characteristic of mitochondrial TyrRSs of fungi belonging to the same subphylum as *N. crassa* and *P. anserina* (Pezizomycotina), and we confirmed that several of these other mitochondrial TyrRSs have group I intron splicing activity (P.J.P. and A.M.L., unpublished observations). These findings suggest that these mitochondrial TyrRSs adapted to function in splicing after the divergence of the Pezizomycotina and the Saccharomycotina about 360 million years ago[26]. A plausible evolutionary model is that the initial interaction was between the nucleotide-binding fold and the P4–P6 domain, and that H0, Ins1 and Ins2 were acquired subsequently to stabilize interactions with the P3–P9 domain, permitting further degeneration of the RNA structure. The conclusion is that an extended scaffold for the group I intron catalytic core developed in multiple steps on a previously unused protein surface in a relatively short period of evolutionary time. The adaptation of an essential mitochondrial TyrRS for group I intron splicing may have been dictated both by tractable features of the protein and by a distinctive genome surveillance mechanism in these fungi, namely repeat-induced point mutations, that effectively prevents functional gene duplications and thereby limits evolutionary options[27]. The fungal mitochondrial TyrRSs now provide a unique model system for studying how essential proteins in general, and aminoacyl-tRNA synthetases in particular, can progressively acquire new functions and evolve to bind multiple structurally related RNAs.

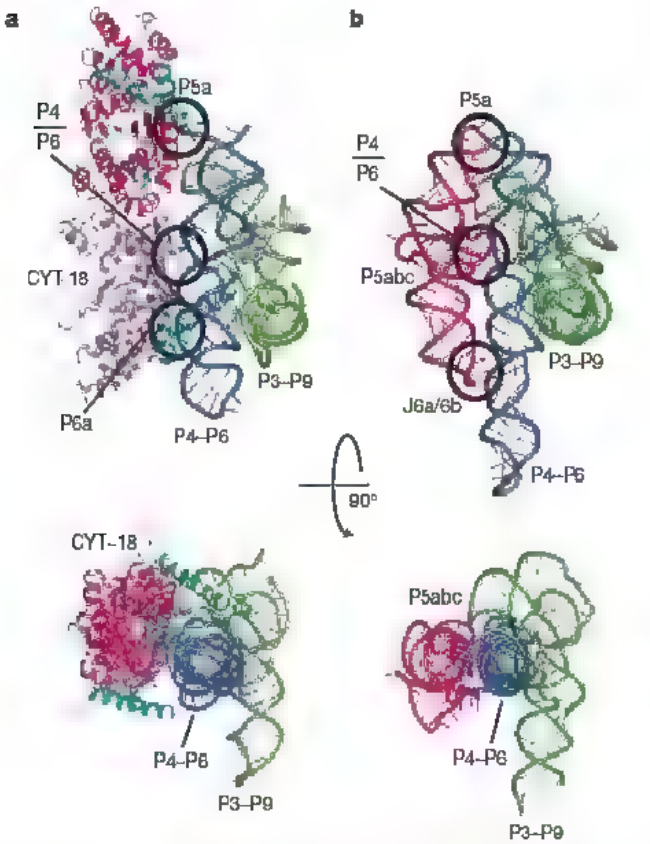


Figure 4 | CYT-18 and the P5abc peripheral RNA structure found in some group I introns interact similarly with the P4–P6 domain. **a**, Orthogonal ribbon diagrams of the CYT-18/Δ424–669–Twort ribozyme co-crystal structure. **b**, The corresponding views of the *T. thermophila* LSU intron crystal structure[6], with the P5abc domain in magenta.

METHODS SUMMARY

Crystallization. The Twort ribozyme and CYT-18/Δ424–669 protein were synthesized and purified as described[7,12], with the addition of a final Superdex 200 gel-filtration step to the protein purification. To renature the Twort ribozyme, 0.06 mM Twort RNA, 0.07 mM RNA product analogue (5′-GCUU,

Dharmacon), 10 mM potassium cacodylate pH 6.5 and 15 mM $MgCl_2$ were heated at 50 °C for 5 min and equilibrated at 18–20 °C for 10 min. CYT-18 protein was added to the RNA such that the molar ratio of dimeric CYT-18/Δ424–669 to RNA was 1:1. Before crystallization, the buffer was exchanged to 10 mM potassium cacodylate pH 6.5, 15 mM $MgCl_2$, 50 mM KCl. Crystals were grown by the hanging-drop method with the use of a well solution of 50 mM potassium cacodylate pH 6.5 and 1.8 M ammonium sulphate.

Data collection and refinement. Data were collected at beamline 23-ID-D at the Advanced Photon Source, and indexed and integrated with HKL2000 (ref. 28) (Supplementary Table 1). Molecular replacement was performed with Phaser[29], with the dimeric CYT-18/Δ424–669 structure[12] and the Twort ribozyme structure[7] as search models (Supplementary Table 2). The model was refined with CNS[30] (Supplementary Table 1), care being taken to minimize potential phase bias (see Supplementary Notes and Supplementary Figs 1 and 7–11).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 26 September; accepted 24 October 2007.

1. Lambowitz, A. M., Caprara, M. G., Zimmerly, S. & Perlman, P. S. in *The RNA World*, 2nd edn (eds Gesteland, R.F., Cech, T.R. & Atkins, J.F.) 451–485 (Cold Spring Harbor Press, New York, 1999).
2. Lambowitz, A. M. & Perlman, P. S. Involvement of aminoacyl-tRNA synthetases and other proteins in group I and group II intron splicing. *Trends Biochem. Sci.* 15, 440–444 (1990).
3. Akins, R. A. & Lambowitz, A. M. A protein required for splicing group I introns in *Neurospora* mitochondria is mitochondrial tyrosyl-tRNA synthetase or a derivative thereof. *Cell* 50, 331–345 (1987).
4. Michel, F. & Westhof, E. Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. *J. Mol. Biol.* 216, 585–610 (1990).
5. Adams, P. L., Stahley, M. R., Kosek, A. B., Wang, J. & Strobel, S. A. Crystal structure of a self-splicing group I intron with both exons. *Nature* 430, 45–50 (2004).
6. Guo, F., Gooding, A. R. & Cech, T. R. Structure of the *Tetrahymena* ribozyme: base triple sandwich and metal ion at the active site. *Mol. Cell* 16, 351–362 (2004).
7. Golden, B. L., Kim, H. & Chase, E. Crystal structure of a phage Twort group I ribozyme–product complex. *Nat. Struct. Mol. Biol.* 12, 82–89 (2005).
8. Mohr, G., Zhang, A., Gianelos, J. A., Belfort, M. & Lambowitz, A. M. The *Neurospora* CYT-18 protein suppresses defects in the phage T4 *td* intron by stabilizing the catalytically active structure of the intron core. *Cell* 69, 483–494 (1992).
9. Guo, Q. & Lambowitz, A. M. A tyrosyl-tRNA synthetase binds specifically to the group I intron catalytic core. *Genes Dev.* 6, 1357–1372 (1992).
10. Mohr, G., Caprara, M. G., Guo, Q. & Lambowitz, A. M. A tyrosyl-tRNA synthetase can function similarly to an RNA structure in the *Tetrahymena* ribozyme. *Nature* 370, 147–150 (1994).
11. Caprara, M. G., Lehnert, V., Lambowitz, A. M. & Westhof, E. A tyrosyl-tRNA synthetase recognizes a conserved tRNA-like structural motif in the group I intron catalytic core. *Cell* 87, 1135–1145 (1996).
12. Paukstelis, P. J. *et al.* A tyrosyl-tRNA synthetase adapted to function in group I intron splicing by acquiring a new RNA binding surface. *Mol. Cell* 17, 417–428 (2005).
13. Yaremchuk, A., Kriklivyi, I., Tukalo, M. & Cusack, S. Class I tyrosyl-tRNA synthetase has a class II mode of cognate tRNA recognition. *EMBO J.* 21, 3829–3840 (2002).
14. Kämper, U., Kück, J., Chermack, A. D. & Lambowitz, A. M. The mitochondrial tyrosyl-tRNA synthetase of *Podospora anserina* is a bifunctional enzyme active in protein synthesis and RNA splicing. *Mol. Cell. Biol.* 12, 499–511 (1992).
15. Kleinjung, J. & Fraternali, F. POPSCOMP: an automated interaction analysis of biomolecular complexes. *Nucleic Acids Res.* 33, W342–6 (2005).
16. Chen, X., Gutell, R. R. & Lambowitz, A. M. Function of tyrosyl-tRNA synthetase in splicing group I introns: an induced-fit model for binding to the P4–P6 domain based on analysis of mutations at the junction of the P4–P6 stacked helices. *J. Mol. Biol.* 301, 265–283 (2000).
17. Caprara, M. G., Myers, C. A. & Lambowitz, A. M. Interaction of the *Neurospora crassa* mitochondrial tyrosyl-tRNA synthetase (CYT-18 protein) with the group I intron P4–P6 domain. Thermodynamic analysis and the role of metal ions. *J. Mol. Biol.* 308, 165–190 (2001).
18. Caprara, M. G., Mohr, G. & Lambowitz, A. M. A tyrosyl-tRNA synthetase protein induces tertiary folding of the group I intron catalytic core. *J. Mol. Biol.* 257, 512–531 (1996).
19. Webb, A. E., Rose, M. A., Westhof, E. & Weeks, K. M. Protein-dependent transition states for ribonucleoprotein assembly. *J. Mol. Biol.* 309, 1087–1100 (2001).
20. Myers, C. A. *et al.* A tyrosyl-tRNA synthetase suppresses structural defects in the two major helical domains of the group I intron catalytic core. *J. Mol. Biol.* 262, 87–104 (1996).
21. Waldsich, C., Grossberger, R. & Schroeder, R. RNA chaperone StpA loosens interactions of the tertiary structure in the *td* group I intron *in vivo*. *Genes Dev.* 16, 2300–2312 (2002).
22. Weeks, K. M. & Cech, T. R. Assembly of a ribonucleoprotein catalyst by tertiary structure capture. *Science* 271, 345–348 (1996).
23. Cate, J. H. *et al.* Crystal structure of a group I ribozyme domain: principles of RNA packing. *Science* 273, 1678–1685 (1996).
24. Chen, X., Mohr, G. & Lambowitz, A. M. The *Neurospora crassa* CYT-18 protein C-terminal RNA-binding domain helps stabilize interdomain tertiary interactions in group I introns. *RNA* 10, 634–644 (2004).
25. Mohr, G., Rennard, R., Chermack, A. D., Stryker, J. & Lambowitz, A. M. Function of the *Neurospora crassa* mitochondrial tyrosyl-tRNA synthetase in RNA splicing: Role of the idiosyncratic N-terminal extension and different modes of interaction with different group I introns. *J. Mol. Biol.* 307, 75–92 (2001).
26. Galagan, J. E., Henn, M. R., Ma, L. J., Cuomo, C. A. & Birren, B. Genomics of the fungal kingdom: insights into eukaryotic biology. *Genome Res.* 15, 1620–1631 (2005).
27. Selker, E. U. Premeiotic instability of repeated sequences in *Neurospora crassa*. *Annu. Rev. Genet.* 24, 579–613 (1990).
28. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* 276, 307–326 (1997).
29. McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C. & Read, R. J. Likelihood-enhanced fast translation functions. *Acta Crystallogr. D Biol. Crystallogr.* 61, 458–464 (2005).
30. Brunger, A. T. *et al.* Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr. D Biol. Crystallogr.* 54, 905–921 (1998).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank C. Correll, M. Hermodson, R. Russell and J. Tesmer for comments on the manuscript; T. Cech for discussions; the staff of SER-CAT and GM/CA-CAT, N. Sanishvili, D. Khare and M. Oldham for assistance with crystallographic data collection, and H. Kim for performing kinetic assays. Data were collected at GM/CA-CAT and SER-CAT beamlines at the Advanced Photon Source, Argonne National Laboratory. This work was supported by a grant from the National Institutes of Health to A.M.L.

Author Contributions P.J.P. and E.C. prepared materials for crystallization, E.C. crystallized the CYT-18–Twort RNA complex, J-H.C. collected and processed diffraction data, P.J.P. solved the structure, P.J.P., B.L.G. and A.M.L. interpreted data and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.M.L. (lambowitz@mail.utexas.edu) or B.L.G. (barbgolden@purdue.edu).

Vol 451|3 January 2008|doi:10.1038/nature06415

LETTERS

Structure of the sulphiredoxin–peroxiredoxin complex reveals an essential repair embrace

Thomas J. Jönsson[1], Lynnette C. Johnson[1] & W. Todd Lowther[1]

Typical 2-Cys peroxiredoxins (Prxs) have an important role in regulating hydrogen peroxide-mediated cell signalling[1]. In this process, Prxs can become inactivated through the hyperoxidation of an active site Cys residue to Cys sulphinic acid. The unique repair of this moiety by sulphiredoxin (Srx) restores peroxidase activity and terminates the signal[2]. The hyperoxidized form of Prx exists as a stable decameric structure with each active site buried. Therefore, it is unclear how Srx can access the sulphinic acid moiety. Here we present the 2.6 Å crystal structure of the human Srx–PrxI complex. This complex reveals the complete unfolding of the carboxy terminus of Prx, and its unexpected packing onto the backside of Srx away from the Srx active site. Binding studies and activity analyses of site-directed mutants at this interface show that the interaction is required for repair to occur. Moreover, rearrangements in the Prx active site lead to a juxtaposition of the Prx Gly-Gly-Leu-Gly and Srx ATP-binding motifs, providing a structural basis for the first step of the catalytic mechanism. The results also suggest that the observed interactions may represent a common mode for other proteins to bind to Prxs.

Reactive oxygen species, such as hydrogen peroxide (H_2O_2) and peroxynitrite, have been recognized as compounds capable of modifying protein, DNA and lipids, especially when present at elevated levels[3]. In contrast, low levels of H_2O_2 can function as a second messenger signal in cell proliferation, differentiation and migration[1,4,5]. The dysregulation of these signalling processes are hallmarks of oxidative stress and disease states, including diabetes, cancer and ageing[3,6]. In this context, the ubiquitous thiol peroxidases, 2-Cys Prxs, function as critical peroxide sensors that can be inactivated through hyperoxidation. The hyperoxidation phenomenon is a fundamental element of the flood-gate hypothesis[7]. Once the Prx molecules are inactivated, through the formation of a Cys sulphinic acid (Cys-$S_PO_2^-$, Fig. 1a) during the catalytic cycle, H_2O_2 can 'breach the gate' to initiate signalling events. Two additional scenarios for Prx-mediated signalling include the sulphinic acid form of 2-Cys Prxs acting as a signal itself and the fostering of disulphide bond formation in other proteins[8,9]. Thus, the unprecedented repair or retroreduction of 2-Cys Prxs by Srx is essential to restore peroxidase activity and the regulation of signalling events.

Structural studies on 2-Cys Prxs have revealed that the active site region can exist in fully folded and locally unfolded states[7,10]. The hyperoxidized form of human PrxII exists in the fully folded state. In this form, the peroxidatic Cys residue, Cys51-$S_PO_2^-$, is located at the amino terminus of an α-helix stabilized by a salt bridge to Arg 127 (Supplementary Fig. 1, residue numbering is one less than in human PrxI) and the resolving Cys-S_RH residue is ~14 Å away[11]. Access to the sulphinic acid moiety is further restricted by the YF and GGLG motifs. The active site helix, however, must locally unfold to allow the formation of a disulphide bond between the Cys-S_PH and Cys-S_RH residues during the Prx catalytic cycle (Fig. 1a). Given these observations, it is clear that large structural rearrangements must occur in order for the Srx molecule to access the Prx sulphinic acid moiety. This notion is supported by the inability to model the catalytic Cys residues of each enzyme in close proximity to each other[12]. Therefore, the complex between the two enzymes is not readily predictable.

Using X-ray crystallography, we determined the structure of human Srx in complex with PrxI to 2.6 Å resolution after screening many engineered constructs. This complex contained one PrxI dimer (Fig. 1b) and two Srx monomers. The electron density across the disulphide bond that bridges between the active sites was unambiguous (Supplementary Fig. 2). The Srx molecules were sandwiched between the active site surface of one PrxI monomer and the C-terminal tail from the adjacent PrxI monomer. Complex formation resulted in the burial of ~690 $Å^2$ at each Srx–Prx active site interface and ~960 $Å^2$ between the C-terminal tail and the 'backside' of Srx (Fig. 1c). Phe 50 of PrxI packs within an Srx pocket (Fig. 2a) constituted by Leu 52, Leu 82, Phe 96, Val 118, Val 127 and Tyr 128 (Supplementary Fig. 3). A comparison to the structure of hyperoxidized human PrxII (Fig. 2b, c) shows that the Cys-SO_2^- moiety (Csd 51) is distant from Srx[11]. We propose that the hydrophobic surface of Srx triggers the local unfolding of the Prx active site helix to place Phe 50 in the Srx pocket. As a result, Cys52-SO_2^- of human PrxI moves ~10 Å away from Arg 128 to approach Cys 99 of Srx.

A superposition of the model of human Srx with ATP bound to it[13], based on the ADP complex determined experimentally, onto the Srx–PrxI complex also suggests that the unfolding of the PrxI active site helix would place Cys52-SO_2^- near the γ-phosphate of ATP (Fig. 2a and Supplementary Fig. 4). In the ternary complex, the γ-phosphate atom is located 3.0 Å from the Sγ atom of PrxI-Cys52 and 3.5 Å from the Sγ atom of Srx-Cys99. The oxygen atom of Cys52-SO_2^- is positioned correctly to perform an inline attack on the γ-phosphate, as originally proposed[2]. In contrast, Cys 99 of Srx points away from the γ-phosphate. This observation implies that this residue does not transfer the phosphate moiety from ATP to the Prx sulphinic acid (Fig. 1a); this is consistent with the weak phosphorylation of the inactive, C99S human Srx variant[14]. The ternary complex also suggests that the GGLG motif and the preceding residues, Lys 92, Lys 93 and Gln 94, are in a position to generate the second half of the ATP binding site.

A comparison of the Srx–PrxI structure to Prx molecules present in two different oxidation states further supports the necessary flexibility of the GGLG motif, the active site helix containing the Cys-S_PH residue, the YF motif, and Cys-S_RH movements. The Srx and the YF motif of the adjacent Prx monomer cannot occupy the same space at the same time (Fig. 2b, c). The active site helix containing the sulphinic acid moiety must break its interaction with a conserved Arg residue (Fig. 2c) in order to attack the ATP molecule in the Srx active site, as described above. This locally unfolded state is consistent with

[1]Center for Structural Biology and Department of Biochemistry, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157, USA

the formation of the disulphide bond between Cys-S_PH and Cys-S_RH (Fig. 2d) during the peroxidase catalytic cycle. The current Srx–PrxI complex also suggests that the ADP molecule would need to be released and that additional structural changes in either of the protein molecules or both would be necessary for Trx or GSH to break down the proposed thiosulphinate intermediate.

The binding of the PrxI C terminus onto the backside of Srx was surprising (Fig. 1). Residues 172–186 of PrxI (Fig. 3a) pack onto a conserved (Supplementary Fig. 5), predominantly hydrophobic groove of Srx. This region of 2-Cys Prxs is also conserved and contains Cys173-S_RH (mutated to Ser in this variant), three or more Pro residues, and Trp 177 (Supplementary Fig. 6). This interface suggests that the active site interactions are insufficient for binding, and that the C terminus functions to hold Srx in the correct orientation for catalysis. In order to test this hypothesis and the relevance of the dimeric complex structure to the Prx hyperoxidized decamer, Ile 50, Tyr 92, Phe 93 and Leu 117 (Fig. 3b) of Srx were mutated to Arg to repel the binding of the Prx C terminus. A corresponding alteration or truncation of the PrxI C terminus was not performed, because Prx variants of these types from a variety of organisms become resistant to hyperoxidation and Cys sulphinic acid cannot be formed[15–17].

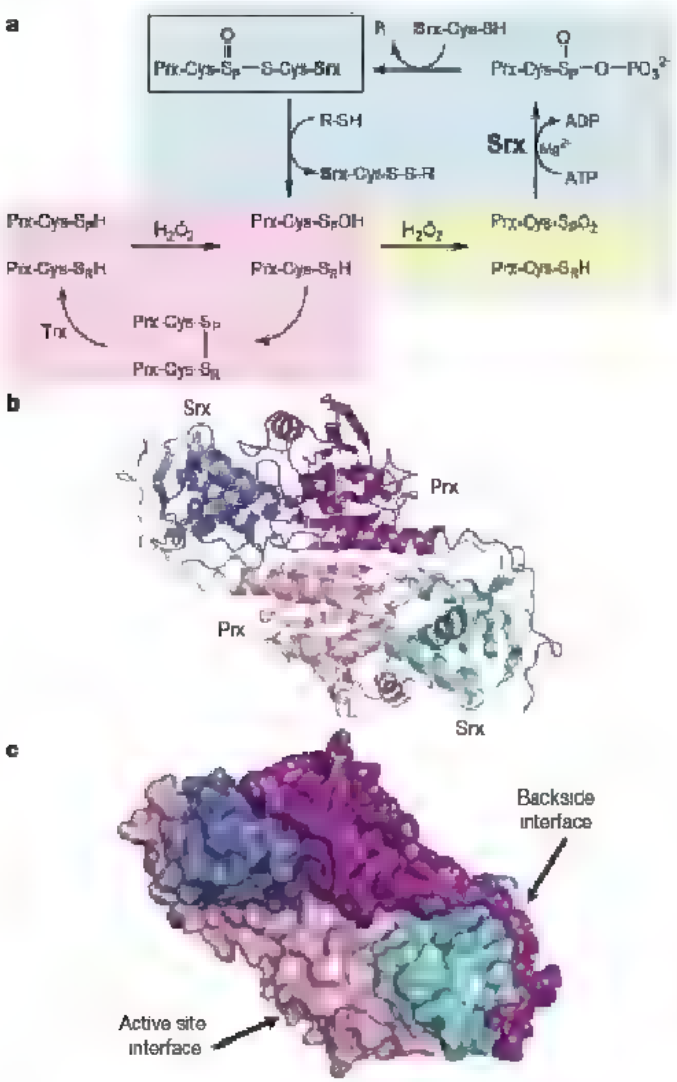


Figure 1 | Peroxiredoxin hyperoxidation and repair by sulphiredoxin. a, In the typical 2-Cys Prx catalytic cycle (violet), the peroxidatic Cys is depicted as a thiol (S_PH) or sulphenic acid (S_POH). The disulphide bond between Cys-S_P and the resolving Cys, Cys-S_RH, from the adjacent monomer is reduced by Trx[10]. Reaction with a second molecule of H_2O_2 results in hyperoxidation and sulphinic acid ($S_PO_2^-$) formation (yellow). The reaction mediated by Srx (blue) is specific for 2-Cys Prxs and dependent upon ATP, Mg^{2+} and a Cys thiol[2,13,14,28]. The sulphinic acid moiety is thought to be phosphorylated to form a sulphinic phosphoryl ester (Prx-$SO_2PO_3^{2-}$) through either a direct attack on the γ-phosphate of ATP or transfer from Cys 99 of human Srx. The phosphoryl ester is subsequently converted to a thiosulphinate bond (for example, Prx-S(O)-S-Srx, boxed in black) which includes the sulphur atom of Cys 99 of human Srx or possibly glutathione (GSH), and inorganic phosphate (P_i) is released. Thioredoxin (Trx) or GSH reduce this complex to release free Srx and Prx-Cys-S_POH. **b**, Overall structure of the Srx–PrxI complex. Cartoon representation is shown, with secondary structural elements along the two-fold axis. The two monomers of PrxI and Srx are shown in violet/purple and cyan/blue, respectively. **c**, Surface representation of the Srx–PrxI complex, illustrating active site and backside interfaces.

The Srx variants were analysed by circular dichroic spectroscopy (Supplementary Fig. 7) to verify that their global structure had not been significantly compromised. Despite the careful selection of the sites of mutation, the F93R mutant exhibited a loss in structure. The ability of the Srx variants, including the partially unfolded F93R variant, to bind wild-type (WT), decameric PrxI-SO_2^- was tested using fluorescence anisotropy (Fig. 3c). WT and I50R Srx bound PrxI-SO_2^- with similar affinities, 5.1 ± 0.9 μM and 7.2 ± 1.3 μM (mean ± s.d.), respectively. This finding agrees with Ile 50 being the residue farthest from the interface. In contrast, the Y92R and L117R variants of Srx had significantly reduced or no binding, a result similar to that of F93R (data not shown). The catalytic activity of the Srx mutants was also monitored using reverse-phase high-performance liquid chromatography (HPLC; Fig. 3d). WT Srx was able to repair decameric PrxI-SO_2^- at a rate of 0.23 min^{-1} (Supplementary Fig. 8), a value similar to the rate previously reported[18]. The I50R and Y92R variants exhibited 60% and 15% of WT activity, respectively. The L117R mutant and the structurally comprised F93R mutant both exhibited no activity. These observations indicate that decreased binding of Srx to Prx is sufficient to reduce or abolish Srx activity.

The necessity for the C terminus of 2-Cys Prxs to bind and embrace Srx highlights its expanding cellular roles. For example, the interaction of the human PrxI C terminus with the PDZ domain of Omi/HtrA2 is necessary to promote protease activity[19]. The interactions with c-Abl, c-Myc, MIF, phospholipase D1 and the PDGF receptor also raise the possibility that the binding of the Pro-rich C terminus of Prx to Srx represents a general mechanism for 2-Cys Prxs to associate with key regulatory or signalling proteins[5,20–22]. Moreover, these latter interactions may modulate the repair process or vice versa.

The importance of Srx is likely to extend beyond the repair of the decameric form of 2-Cys Prxs. The association of Prx decamers into stacks of toroids has been observed via electron microscopy and within the crystal structure of human PrxII-SO_2^- (refs 11, 23). Confocal microscopy studies also suggest that human PrxII-SO_2^- can form filamentous structures in cell culture, thereby alerting cells to a perturbation in peroxide homeostasis[24]. Sphere-like Prx aggregates have also been shown to switch from a peroxidase activity to a protein chaperone function[25]. In an effort to understand how Srx may interact with the higher-order forms of Prxs, a model of the decameric Srx–PrxI complex was generated. The PrxI dimer of the Srx–Prx complex was superimposed onto each of the five Prx dimers of the PrxII-SO_2 structure (Supplementary Fig. 9). No significant steric clashes were observed, suggesting that the Srx–Prx interaction is not influenced by the oligomeric state. The addition of ten Srx molecules, however, did expand the toroid diameter (~110 to 125 Å) and thickness (~45 to 55 Å). These substantial changes suggest that the binding of one or two Srx molecules would be sufficient to destabilize Prx–Prx interactions in higher-order oligomers.

In summary, the embrace observed in the Srx–Prx complex represents an unexpected structural rearrangement fundamentally important for the repair of Prxs in higher organisms. A structural basis is now available for designing future biochemical and cellular studies to dissect additional aspects of the Srx reaction mechanism and the roles of Srx and Prxs in modulating cell signalling.

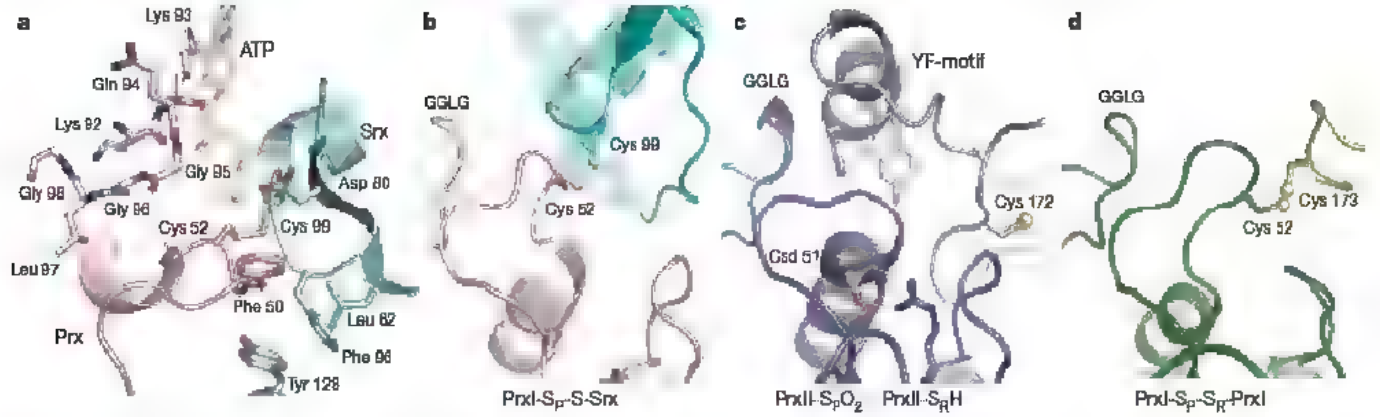


Figure 2 | Srx—PrxI active site interactions and structural plasticity. **a**, ATP modelled (translucent) into the active site of the Srx–PrxI complex containing the disulphide bond between Cys 99 of Srx (cyan) and Cys 52 of PrxI (violet). **b**, Ribbon diagram of the locally unfolded human PrxI active site in complex with Srx. **c**, Human PrxII-SO_2^- active site in the fully folded state (dark purple) with the YF motif from the adjacent monomer (light purple) overlaying the active site. The active site Cys-S_PH residue is in the sulphinic acid form (Csd 51). PDB code 1QMV[31]. Human PrxI and PrxII exhibit 77 8% sequence identity. **d**, Rat PrxI active site (dark green) present in the oxidized, disulphide form involving the resolving Cys residue, Cys 173 (light green). PDB code 1QQ2[27]. Panels **b–d** are presented in the same orientation using a superposition of the core β-sheet structure of each Prx dimer.

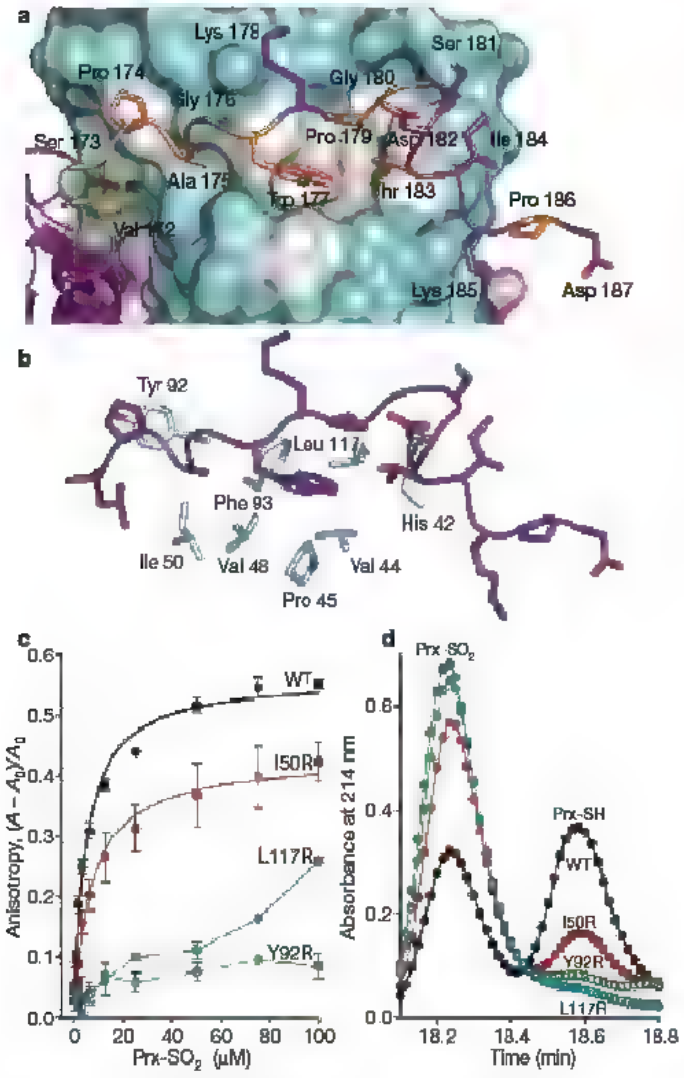


Figure 3 | PrxI backside interaction with Srx. **a**, The C-terminal tail of PrxI binds to Srx. Conserved residues of PrxI are coloured orange, and the conserved surface of Srx is coloured grey. **b**, Surface residues of Srx that interact with the C terminus of PrxI. **c**, Srx–PrxI-SO_2^- interactions measured in solution by changes in fluorescence anisotropy with Oregon Green 514-labelled Srx variants. Data obtained from representative, duplicate titrations are expressed as the fractional change in anisotropy, $(A–A_0)/A_0$, versus the concentration of decameric PrxI-SO_2^- added, with the error bars indicating s.d. The data for WT Srx and the I50R mutant were fitted to a single-site, saturable binding model. **d**, Representative HPLC traces from the activity analysis of Srx variants.

METHODS SUMMARY

One key to stabilizing the Srx–Prx crystals was to mimic the proposed thiosulphinate intermediate (Fig. 1a) with a disulphide bond. An intermolecular disulphide bond was formed between the active site residues, Cys 99 of human Srx and Cys52-S_PH of the C71S, C83E, C173S variant of PrxI. Both proteins were separately overexpressed in *Escherichia coli*, and disulphide bond formation was facilitated by pre-treatment of the PrxI variant with 5,5′-dithiobis-(2-nitrobenzoic acid). The comparable disulphide-bonded species has also been observed *in vivo* and *in vitro*[2,14]. A dimeric form of PrxI was also necessary, and was generated by introducing charged residues, Cys83Glu on each monomer, juxtaposed at the dimer–dimer interface of the PrxI decamer[26]. An N-terminal truncation of human Srx, residues 1–37, was required to remove a non-conserved, glycine-rich region[13]. Crystals were grown by vapour diffusion, and diffraction data collected on beamline X8C at the National Synchrotron Light Source (NSLS). The structure was solved by molecular replacement using the rat PrxI dimer and human Srx as search models[13,27]. The final model has R_{work} and R_{free} values of 23.9% and 30.8%, respectively. The binding of Srx variants to the hyperoxidized, decameric form of human PrxI was determined by fluorescence anisotropy by labelling Srx with Oregon Green 514. Hyperoxidized PrxI was generated by forcing the enzyme to go through the catalytic cycle many times by the addition of H_2O_2 and dithiothreitol. The activity of Srx variants was determined by quantifying the conversion of Prx-SO_2 to Prx-SH by reverse-phase HPLC. Detailed procedures are presented in Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 20 June; accepted 24 October 2007.

1. Rhee, S. G. Cell signaling. H_2O_2, a necessary evil for cell signaling. *Science* 312, 1882–1883 (2006).
2. Biteau, B., Labarre, J. & Toledano, M. B. ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. *Nature* 425, 980–984 (2003).
3. Finkel, T. & Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247 (2000).
4. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K. & Finkel, T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270, 296–299 (1995).
5. Kang, S. W., Rhee, S. G., Chang, T. S., Jeong, W. & Choi, M. H. 2-Cys peroxiredoxin function in intracellular signal transduction: Therapeutic implications. *Trends Mol. Med.* 11, 571–578 (2005).
6. Klaunig, J. E. & Kamendulis, L. M. The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 44, 239–267 (2004).
7. Wood, Z. A., Poole, L. B. & Karplus, P. A. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300, 650–653 (2003).
8. Bozonet, S. M. *et al.* Oxidation of a eukaryotic 2-Cys peroxiredoxin is a molecular switch controlling the transcriptional response to increasing levels of hydrogen peroxide. *J. Biol. Chem.* 280, 23319–23327 (2005).
9. Vivancos, A. P. *et al.* A cysteine-sulfinic acid in peroxiredoxin regulates H_2O_2-sensing by the antioxidant Pap1 pathway. *Proc. Natl Acad. Sci. USA* 102, 8875–8880 (2005).
10. Wood, Z. A., Schröder, E., Harris, R. J. & Poole, L. B. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem. Sci.* 28, 32–40 (2003).

11 Schröder, E. *et al.* Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes at 1.7 Å resolution. *Structure* **8**, 605–615 (2000).
12. Lee, D. Y. *et al.* Mutagenesis and modeling of the peroxiredoxin (Prx) complex with the NMR structure of ATP-bound human sulfiredoxin implicate aspartate 187 of Prx I as the catalytic residue in ATP hydrolysis. *Biochemistry* **45**, 15301–15309 (2006).
13. Jönsson, T. J., Murray, M. S., Johnson, L. C., Poole, L. B. & Lowther, W. T. Structural basis for the retroreduction of inactivated peroxiredoxins by human sulfiredoxin. *Biochemistry* **44**, 8634–8642 (2005).
14. Jeong, W., Park, S. J., Chang, T. S., Lee, D. Y. & Rhee, S. G. Molecular mechanism of the reduction of cysteine sulfinic acid of peroxiredoxin to cysteine by mammalian sulfiredoxin. *J. Biol. Chem.* **281**, 14400–14407 (2006).
15. Koo, K. H. *et al.* Regulation of thioredoxin peroxidase activity by C-terminal truncation. *Arch. Biochem. Biophys.* **397**, 312–318 (2002).
16. Moon, J. C. *et al.* Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H_2O_2-induced cell death. *J. Biol. Chem.* **280**, 28775–28784 (2005).
17. Sayed, A. A. & Williams, D. L. Biochemical characterization of 2-Cys peroxiredoxins from *Schistosoma mansoni*. *J. Biol. Chem.* **279**, 26159–26166 (2004).
18. Chang, T. S. *et al.* Characterization of mammalian sulfiredoxin and its reactivation of hyperoxidized peroxiredoxin through reduction of cysteine sulfinic acid in the active site to cysteine. *J. Biol. Chem.* **279**, 50994–51001 (2004).
19. Hong, S. K., Cha, M. K. & Kim, I. H. Specific protein interaction of human Pag with Omi/HtrA2 and the activation of the protease activity of Omi/HtrA2. *Free Radic. Biol. Med.* **40**, 275–284 (2006).
20. Choi, M. H. *et al.* Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* **435**, 347–353 (2005).
21. Jung, H., Kim, T., Chae, H. Z., Kim, K. T. & Ha, H. Regulation of macrophage migration inhibitory factor and thiol-specific antioxidant protein PAG by direct interaction. *J. Biol. Chem.* **276**, 15504–15510 (2001).
22. Xiao, N., Du, G. & Frohman, M. A. Peroxiredoxin I functions as a signal terminator for H_2O_2-activated phospholipase D1. *FEBS J.* **272**, 3929–3937 (2005).
23. Harris, J. R. *et al.* Comparison of the decameric structure of peroxiredoxin-II by transmission electron microscopy and X-ray crystallography. *Biochim. Biophys. Acta* **1547**, 221–234 (2001).
24. Phalen, T. J. *et al.* Oxidation state governs structural transitions in peroxiredoxin II that correlate with cell cycle arrest and recovery. *J. Cell Biol.* **175**, 779–789 (2006).
25. Jang, H. H. *et al.* Two enzymes in one: Two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. *Cell* **117**, 625–635 (2004).
26. Parsonage, D. *et al.* Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. *Biochemistry* **44**, 10583–10592 (2005).
27. Hirotsu, S. *et al.* Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. *Proc. Natl Acad. Sci. USA* **96**, 12333–12338 (1999).
28. Woo, H. A. *et al.* Reduction of cysteine sulfinic acid by sulfiredoxin is specific to 2-Cys peroxiredoxins. *J. Biol. Chem.* **280**, 3125–3128 (2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank M. Murray for contributions to the structure determination, L. B. Poole, P. A. Karplus and N. H. Heintz for discussions, the staff of the NSLS and beamline X8C for their assistance during data collection and the RapiData course, and R. R. Hantgan for help with the circular dichroism and fluorescence anisotropy experiments. This work was supported by an NIH grant (W.T.L.) and an American Heart Association Postdoctoral Fellowship (T.J.J.). NSLS is supported by the US Department of Energy and NIH.

Author Contributions T.J.J. and L.C.J. performed all biochemical and crystallization experiments. T.J.J. and W.T.L. solved the structure. T.J.J. and W.T.L. wrote the paper. All authors discussed the results and commented on the manuscript.

Author Information Coordinates and structure factors have been deposited with the Protein Data Bank under the accession number 2RII. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to W.T.L. (lowther@wfubmc.edu).

CORRIGENDUM

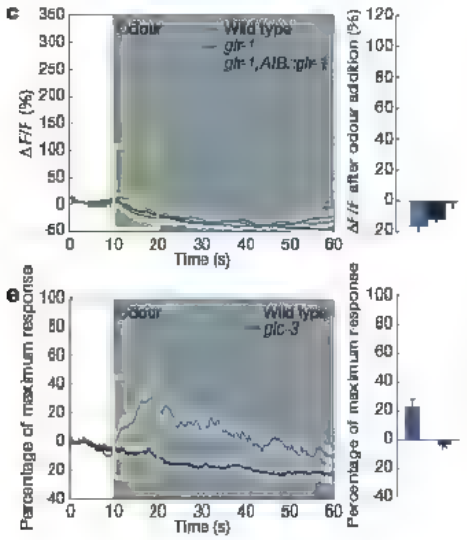
doi:10.1038/nature06540

Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*

Sreekanth H. Chalasani, Nikos Chronis, Makoto Tsunozaki, Jesse M. Gray, Daniel Ramot, Miriam B. Goodman & Cornelia I. Bargmann

Nature 450, 63–70 (2007)

In parts of Fig. 5c and e of this Article, incorrect data were inadvertently used. The corrected figure panels are shown below. Our results and conclusions are not affected.



CORRIGENDUM

doi:10.1038/nature06541

Gene-specific control of inflammation by TLR-induced chromatin modifications

Simmie L. Foster, Diana C. Hargreaves & Ruslan Medzhitov

Nature 447, 972–978 (2007)

A citation (ref. 1) was inadvertently removed during revision of this Article, which also emphasized the potential importance of chromatin modifications in innate immune responses.

1. Chan, C., Li, L., McCall, C. E. & Yoza, B. Endotoxin tolerance disrupts chromatin remodeling and NF-κB transactivation at the IL-1β promoter. *J. Immunol.* 175, 461–468 (2005).

Advertisement feature

Liquid Assets

Time is money

It's 2008. In today's pharmaceutical industry, time is one of the most important assets. Researchers must be able to quickly test enormous sample amounts for leads. The tools below can help you save precious time.

image supplied by Tecan

The SFT-250 Supercritical Fluid Extraction / Reaction (SFE/SFR) Processing System, from Activotec, is designed to perform extractions and run reactions in a range of supercritical fluid media. The SFT-250 is simple to operate, easy to modify for evolving application needs, and features many of the performance characteristics of fully automated pilot scale systems. The SFT-250 offers operational flexibility and safety features unmatched by any other laboratory SFE/SFR instrument available in the market today. At the heart of the SFT-250 is a stainless steel vessel capable of containing supercritical fluids at pressures up to 10,000 psi (68.9mPa). The SFT-250 accommodates pressure vessels of up to 4 liters in size. The SFT-250 incorporates a high performance, air driven pump, which can rapidly produce the high pressures required for supercritical fluid work. Under typical operating conditions, flow rates range from 1 to 330mL/min (250 grams/min) of liquid CO_2. Conveniently located front panels allow easy access to the pressure vessel, valves, fittings, and electronics. Manually actuated valves provide long term, maintenance free performance.

Anachem introduces the next generation of peristaltic pump, the **Gilson Minipuls Evolution.** This patented new design includes unique cartridges that snap in and out of the horizontal pump head without the use of any tool, enabling the fastest, easiest tubing changing even when the pump is running. Together with the intuitive software interface and a large LCD screen, set up is quick, flexible and safe. The Minipuls Evolution will expand to accommodate your changing workload with stackable pump heads providing up to 16-channels. The Minipuls Evolution has a flow rate range from 0.2µl/min to 340mL/min with an optimized 6 roller design which limits pulsations, offering one of the widest and constant volume ranges available. With 0.01rpm readability below 10rpm, you benefit from accurate flow rate adjustments and guaranteed peace of mind.

Beckman Coulter announces the **BioRAPTR and PicoRAPTR** liquid handling systems, developed by Aurora Discovery, featuring advanced, accurate fluidic technology ideal for assay miniaturization. The BioRAPTR workstation is an automated, non-contact dispenser for 384-, 1536- and 3456-well plate formats. It delivers precise and accurate dispensing across a low-volume range of 100nl to 60µl, without cross contamination. The PicoRAPTR features an eight-tip piezo head and dual-plate carrier stage for high-speed aspiration and pipetting of ultra-low volumes – 1nl to 100µl. With various configurations, the PicoRAPTR workstation provides the ability to complete spotting; compound formatting; dose response for cell-based assays; and SNP assays in high-density microplates, slides and chips.

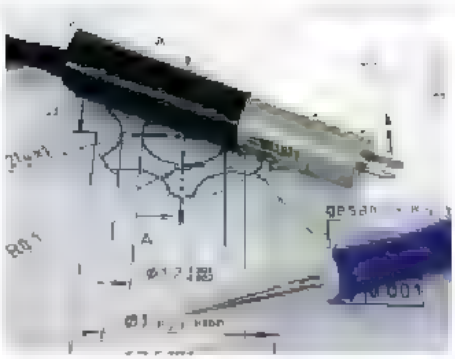
The **Multipette stream** and **Multipette Xstream, from Eppendorf,** are the world's first handheld dispensers with an easy-to-read illuminated display. The display ensures clear visibility in every environment. The Multipette stream is suitable for all standard pipetting and dispensing applications. Additiona. functions, such as titration and aspirating of supernatants, can be performed with the Multipette Xstream. Together with Combitips Plus pipette tips, Multipette stream and Xstream make a perfect and essential system for every lab. Multipette stream and Xstream won the red dot design award for their innovative and customer friendly design.

A new range of **Micro-Dispense Nozzles** which provide a convenient method of accurately controlling dispensed volumes of liquid in the microliter to nanoliter range,

Material compiled by
Kenyon Hoag Associates

KENYON
HOAG
ASSOCIATES
Your Expert in Marketing to the World of Science.
www.kenyonhoag.com

Beckman Coulter liquid handling systems



Micropump MZR series from Michael Smith Engineers

Thermo Scientific Multidrop Combi nl from Thermo Fisher Scientific

are now available through miniature component specialists, Lee Products. Lee Micro-Dispense Nozzles are designed to be used in conjunction with Lee VHS (Very High Speed) Micro Dispense Solenoid Valves, a combination which provides users with a range of features and benefits. They are available in two different mounting configurations to enable optimum system design flexibility, 062 MINSTAC threaded ends and straight tube. Nozzles with 062 MINSTAC ends are designed to be threaded directly into Lee VHS valves equipped with 062 MINSTAC outlet bosses.

Sarstedt announces several improvements to its line of vacuum unit and bottle top filters. All filters now contain a low binding, fast throughput PES membrane with a pore size of either 0.22µm or 0.45µm. Lot number, membrane material, and pore size are conveniently imprinted onto each filter housing. The redesigned ported neck is compatible with 45mm receiving bottles and is securely attached to the filter housing for an improved connection. Vacuum units are assembled with new ergonomic receiving bottles that feature a wide base for stability and two opposing indented sections for improved grip and handling. Sarstedt vacuum units, bottle top filters, and receiving bottles are available in 250ml and 500ml volume options. All items are individually wrapped, sterile, and certified non-pyrogenic and non-cytotoxic.

Thermo Fisher Scientific introduces the **Thermo Scientific Multidrop Combi nl** bulk reagent dispenser for nanoliter-to-microliter volumes. The Multidrop Combi nl provides accurate, consistent, high-throughput dispensing for all laboratories across a volume range of 50nl to 50µl. The Multidrop Combi nl is easy to maintain and can be used to dispense all common reagents, diluents, buffers and solvents as well as viscose solutions, cells and beads, making it ideal for the large variety of assays and protocols. The Thermo Scientific Multidrop Combi nl uses a pressurized reagent container with valves to provide high precision and accurate control of liquid flow at all volumes. As a result, reagent waste is greatly decreased, minimizing costs and maximizing the utilization of precious samples. The intuitive graphical user interface makes all functions easy to set-up and use. The PC software control option enables greater flexibility, functionality and easier protocol creation. Furthermore, protocols can be downloaded onto the instrument for standalone use.

VICI Valco's Cheminert Model M6 and **M50** liquid handling pumps are a new generation of pumps for precision handling of liquids and/or gases, producing a bidirectional pulse-less flow with a range of over six orders of magnitude (10nl/min to 10ml/min for the M6 Pump; 50µl/min to 50 ml/min for the M50 Pump). An excellent replacement for syringe pumps, the Cheminert pumps offer better performance and eliminate the need for refill cycles and syringe changes. The M6 and M50 are positive displacement pumps, which means they are self-priming and tolerant of any gas which may find its way into the fluid lines. There is no separate fill cycle, and the capacity is unlimited.

The **Micropump MZR series** of micro annular gear pumps, from **Michael Smith Engineers**, are ideal for precision liquid handling duties because of their compact design, which integrates pump, drive, control, and a precise close tolerance machining of the pump components. MZR pumps are offered in two series of pumps which cover flow rates from 0.15ml/min up to 300ml/min and discrete individual dispense amounts starting from as little as 0.25µl. These small precise pumps are hard wearing and give accurate pulse free pumping for long periods between servicing. All the pumps are available with precision DC drives which ensure a dosing accuracy of better than 1%.

The **Freedom EVO series**, from Tecan, offers versatile liquid handling platforms and flexible robotic workstations serving a full range of life science applications. The Freedom EVO platform delivers flexibility and possibilities for expansion according to future needs. Powered by a new intuitive software, Freedom EVOware, each system is equipped to successfully automate genomic, proteomic, drug discovery, and other life science applications. Tecan offers the Freedom EVO platform in four different base sizes (75, 100, 150 and 200cm). Each platform can be combined with a wide choice of robotic arms, liquid handling tools and application options powered by a straightforward software to meet individual needs, so now all laboratory personnel have access to a platform that will advance with their application needs.

Companies mentioned in this Product Focus

Activ[illegible]
Anachem – www.anachem.com
Beckman Coulter – www.beckmancoulter.com
Eppendorf – www.eppendorf.com
Lee Products – www.theleeco.com
Michael Smith Engineers –
www.michael-smith-engineers.co.uk
Sarstedt – www.sarstedt.com
Tecan – www.tecan.com
Thermo Scientific – www.thermo.com
VICI Valco – www.vici.com

"This article was compiled by Kenyon Hoag Associates and submitted to Nature. It has not been written by or reviewed by the Nature editorial team and Nature takes no responsibility for the accuracy or otherwise of the information provided. Submit press releases for consideration to productfocus@nature.com with the topic in the subject line."

Clinical Pharmacology & Therapeutics

WOMEN'S HEALTH

Clinical Pharmacology & Therapeutics is expanding coverage in 2008. Original articles, Letters to the Editor, and Commentaries, alongside crucial research, deliver issues that are important to you.

The **Women's Health** issue features:

Point-counterpoint: Emergency contraception
Emergency contraception: a reasonable personal choice or a destructive societal influence?
M Greene, et al
Emergency contraception: an overestimated effectiveness and questionable expectations
J Stanford, et al

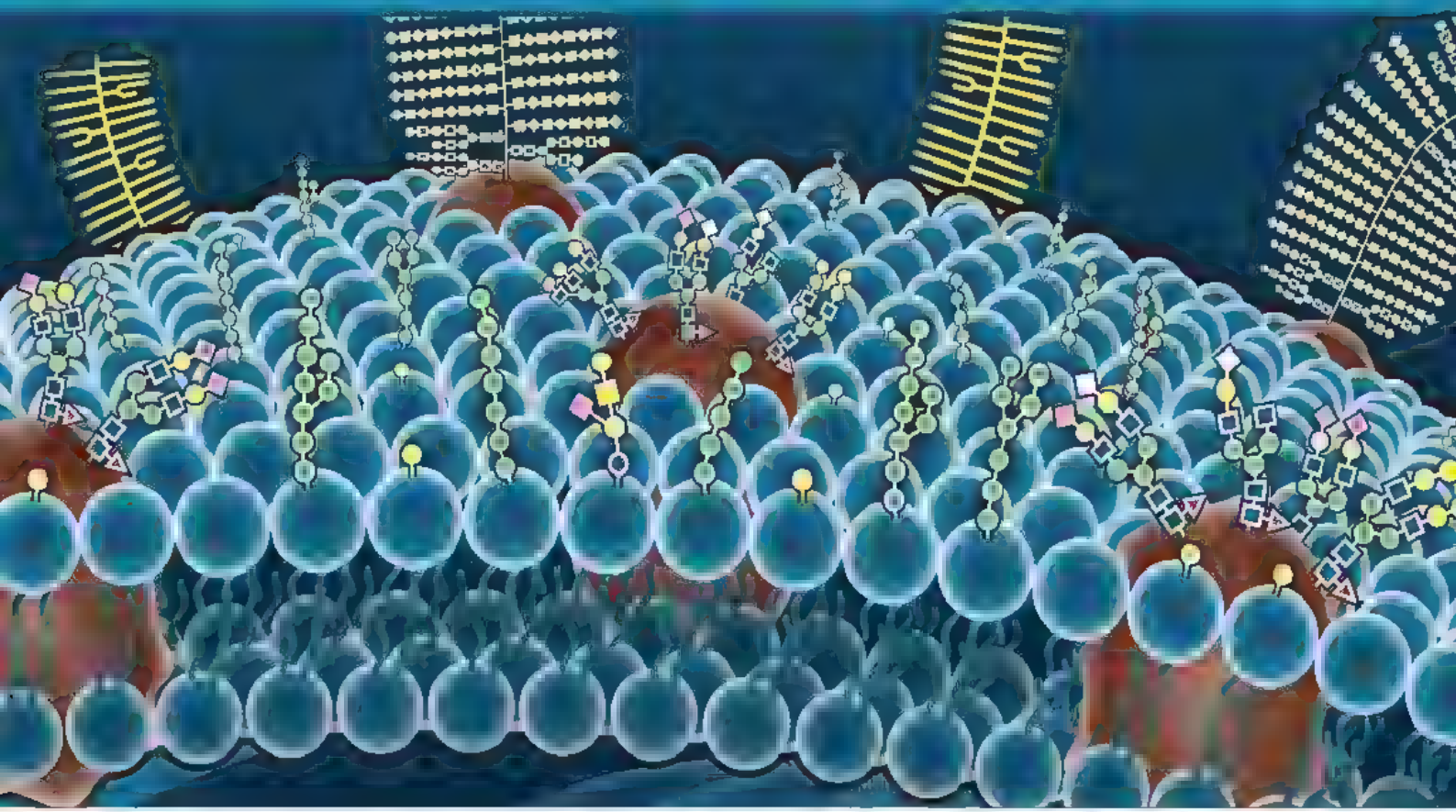
State of the Art:
Advances in the treatment of breast cancer
G Hortobagyi, et al

Practice:
Emergency contraception update: a Canadian perspective
J Soon, et al

Visit **www.nature.com/cpt** to view select free articles from this issue and discover the impact the findings are having on today's woman.

CFG

nature functionalglycomicsgateway



sweet spots in biology

- research highlights
- news & events
- databases, data & reagents
- glycan-binding proteins
- carbohydrate structures
- glycan expression profiles

and more

sign-up for email alerts

Free online at

www.functionalglycomics.org

A collaboration between the Consortium for Functional Glycomics & Nature Publishing Group

nature publishing group npg

Assistant Professor, Petroleum Microbiology

UNIVERSITY OF CALGARY

The **Department of Biological Sciences** at the **University of Calgary** invites applications for a tenure-track Assistant Professor position in Petroleum Microbiology. This position was made possible through the recently established NSERC Industrial Research Chair in Petroleum Microbiology (www.bio.ucalgary.ca/contact/faculty/voordouw.html), a program supported by eight industrial partners, the Alberta Energy Research Institute (AERI) and the University of Calgary. The position is intended to complement existing strengths and further enhance our international reputation in this area.

Applicants who have used molecular and/or physiological approaches to study a relevant problem in applied and environmental microbiology are encouraged to apply. Willingness to work in petroleum microbiology, especially on microbial communities and processes in oil sands environments such as outcrops, tailings ponds, as well as surface and subsurface bitumen formations, is expected. Although the successful candidate will help achieve the research goals of the Industrial Research Chair program, every effort will be made to ensure he/she pursues a successful independent career in the target area.

Applicants must have a PhD, at least one year of relevant post-doctoral experience and an established record of high-quality research. In addition to pursuing a successful, externally funded research program in petroleum microbiology, the successful candidate is expected to help develop an interdisciplinary course in the area and participate in undergraduate and graduate teaching. The Department of Biological Sciences (www.bio.ucalgary.ca) is a large and diverse department with excellent facilities. Interdisciplinary and resource opportunities exist in the newly established Institute for Sustainable Energy, Environment, and Economy (www.iseee.ca) and the Institute of Biocomplexity and Informatics (www.ibi.ucalgary.ca).

The City of Calgary (pop. 1 million) is the Centre of Canada's Energy Industry. It has a lively cultural life, vibrant economy and many recreational opportunities. It is located less than an hour from the Rocky Mountains and Banff National Park.

Applications should include a covering letter that clearly specifies the interest in and qualifications for the offered position, a curriculum vitae, a concise outline of research plans, copies of up to five publications, a statement of teaching interests and philosophy, and three letters of reference sent directly by the referees. The deadline for receipt of a complete application package is **March 15, 2008.** Applications should be sent to:

Dr. Jeffrey I. Goldberg, Head
Department of Biological Sciences, University of Calgary
2500 University Drive N.W. Calgary, Alberta, T2N 1N4
Fax: (403) 289-9311

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. The University of Calgary respects, appreciates, and encourages diversity.

To see all career opportunities available at the University of Calgary, please visit **www.ucalgary.ca/hr/careers**.

NW121699R

PERIMETER INSTITUTE

DIRECTOR OF OUTREACH

NW120963R

Canada's Perimeter Institute is a premier centre for scientific research and educational outreach devoted to foundational issues in theoretical physics at the highest levels of international excellence. To complement an ambitious research agenda, the Institute has developed a world-class outreach program to disseminate the mysteries of the physical world to students, teachers and the general public.

The Institute is seeking a highly experienced strategic manager with the ability, ambition and vision to establish PI as a leader in physics outreach internationally.

The Director of Outreach will be responsible for overseeing all outreach programs and products, managing the logistics and finances of numerous large scale projects; leading staff in the planning, development, and delivery of new and existing initiatives; and identifying potential targets of opportunities for new programs and products.

The successful candidate will have a passion for outreach, a strong background and education in science, and a history of developing and implementing innovative community and educational programs and products.

Perimeter Institute offers a dynamic work environment and competitive salary and benefits. Highly qualified candidates wishing to be considered for the position may apply by sending a cover letter and curriculum vitae by email to: **hr@perimeterinstitute.ca** Please quote Competition #20071210 in the subject line of your email. The deadline for applications is January 31, 2008.

For further insight into this unique opportunity please visit our website at **www.perimeterinstitute.ca**

We are an equal opportunity employer. All qualified candidates are encouraged to apply; however, Canadian citizens and Permanent Residents will be given priority.

www.perimeterinstitute.ca

FACULTY POSITION

Genetics/Skeletal Biology

The Department of Genetics at Harvard Medical School and the Department of Orthopaedic Surgery at Children's Hospital Boston invite applications for a tenure-track appointment at the Assistant Professor level. The appointee will be a full member and active participant in the Department of Genetics (**http://genetics.med.harvard.edu**) and be given laboratory space in the Orthopaedic Research Laboratories at Children's Hospital Boston (**http://www.childrenshospital.org**).

The successful candidate is expected to direct innovative and independent research in the field of skeletal biology, preferably addressing questions related to pediatric skeletal health and disease, and to participate in the teaching activities of the Department of Genetics and the Department of Orthopaedic Surgery. Applicants must hold a Ph.D., M.D./Ph.D., or M.D. degree, have completed post-doctoral training, and have a strong record of research accomplishments.

Applicants should submit electronically (pdf format only) a curriculum vitae, bibliography, and a 3-page description of research accomplishments/future research interests by February 29, 2008, and ask 3 references to provide letters of recommendation (also in pdf format). All these materials should be sent to the following email address:

faculty_search@genetics.med.harvard.edu

Harvard Medical School and Children's Hospital Boston are Equal Opportunity/Affirmative Action Employers. Applications from women and minorities are encouraged.

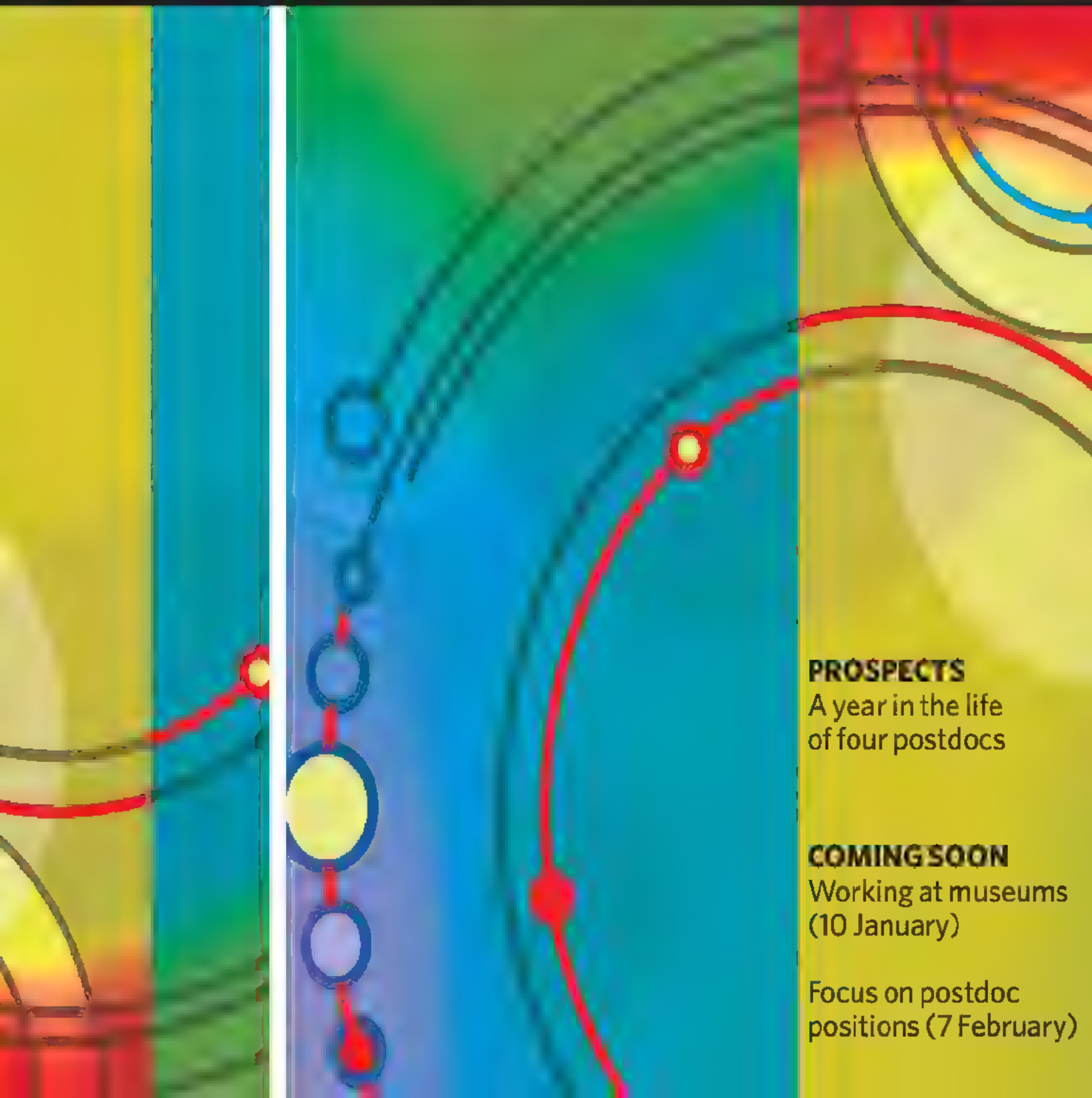
NW121348R

NATURE|Vol 451|3 January 2008

THE CAREERS MAGAZINE FOR SCIENTISTS

- FOCUS
- SPOTLIGHT
- RECRUITMENT
- ANNOUNCEMENTS
- EVENTS

nature jobs



PROSPECTS
A year in the life of four postdocs

COMING SOON
Working at museums (10 January)

Focus on postdoc positions (7 February)

naturejobs

Any decent novel has an interesting narrative arc. The reader gets to know the characters in intimate detail and, by the story's end, at least one character changes in some intriguing, enlightening or surprising way. The intention is the same with our Postdoc Journal. Over the past 11 months, readers have seen changing moods, outlooks, opinions, attitudes and priorities from our four journal keepers — changes that, we hope, reflect readers' own concerns

As the tenure of our 2007 journal keepers comes to an end, our four narrative arcs are summed up in online-only end-of-the-year entries, one from each journal keeper (see www.nature.com/naturejobs/magazine/graduates/index.html). Chris Rowan began the year with a risky move from Britain to South Africa to do a postdoc in geology. He couldn't resist the opportunity — or the area's unusual geological formations. But like so many postdocs, he is concerned about publishing and finding a niche that will help him launch an independent career. He also misses teaching, although so far he has no regrets about his move.

Maria Ocampo-Hafalla came to appreciate more than ever her partnerships, both personal and professional. She writes that finding a good mentor, along with being resilient with experiments, having a clear professional vision and "developing skills for scientific survival", are among the ingredients in her recipe for career success. Moira Sheehan began the year with a joy and challenge many can relate to: a new child. Daycare, illnesses, balancing lab responsibilities with kids — Sheehan was very honest about her continued struggles. And, she says, readers responded with their own stories. Personal distractions have so far made it hard for Sheehan to make big professional decisions — maybe industry, maybe a small liberal arts college.

For Peter Jordan, the year helped him come to a realization about his future in science research: he decided he didn't have one. Although his time in the lab has been far from a nightmare, after a year as a postdoc, Jordan has elected to move on to other pursuits. He wants a job that has more of a direct impact on people. Jordan, like the others, has changed a bit in the past year. The narrative arcs continue.

Gene Russo, acting editor of *Naturejobs*

CONTACTS
Acting Editor: Gene Russo

US Head Office
New York
75 Varick Street,
9th Floor,
New York,
New York 10013-1917
Tel: +1 800 989 7718
Fax: +1 800 989 7109
e-mail: naturejobs@natureny.com

US Sales Manager/Corporations:
Peter Biess
Tel: +1 800 989 7718

San Francisco Office
Classified Sales Representative:
Michaela Bjorkman
West USA/West Corp. Canada
225 Bush Street, Suite 1453
San Francisco,
California 94104
Tel: +1 415 781 3803
Fax: +1 415 781 3805
e-mail: m.bjorkman@naturesf.com

European Head Office
London
The Macmillan Building,
4 Crinan Street,
London N1 9XW, UK
Tel: +44 (0) 20 7843 4961
Fax: +44 (0) 20 7843 4996
e-mail: naturejobs@nature.com

European Sales Manager:
Andy Douglas (4975)
Advertising Production Manager:
Stephen Russell
To send materials use London address above.
Tel: +44 (0) 20 7843 4816
Fax: +44 (0) 20 7843 4996
e-mail: naturejobs@nature.com

***Naturejobs* web development:**
Tom Hancock

***Naturejobs* online production:**
Jasmine Myer

Japan Head Office
Tokyo
Chiyoda Building,
2-37 Ichigayatamachi,
Shinjuku-ku,
Tokyo 162-0843
Tel: +813 3267 8751
Fax: +81 3 3267 8746

Asia-Pacific Sales Manager:
Ayako Watanabe
Tel: +81-3-3267-8765
e-mail: a.watanabe@natureasia.com

JOBS OF THE WEEK

Faculty Position
Harvard Medical School/Children's Hospital in Boston
Boston, MA (USA)
Turn to page IFC

PhD Studentships
MRC
Cambridge (UK)
Turn to page 7

Associate Professor (W3)
DKFZ
Heidelberg (Germany)
Turn to page 104

Chair
University of Pittsburgh - School of Medicine
Pittsburgh, PA (USA)
Turn to page 10

Director
UCL
London (UK)
Turn to page 6

dkfz.

German Cancer Research Center
(DKFZ, Deutsches Krebsforschungszentrum) Member of the Helmholtz-Gemeinschaft, HGF

Medical Faculty University of Heidelberg

The Department of Translational Oncology of the National Center for Tumor Diseases (NCT) Heidelberg in collaboration with the German Cancer Research Center and the University of Heidelberg Faculty of Medicine invite applications for the position of an

Associate Professor (W3)

in

Translational Hematology and Oncology

This position also entails heading a section of the Division of Translational Oncology.

We are seeking a colleague who shares our commitment to effectively translate advances in basic research in hematology and oncology and newly gained insight into the pathogenesis of tumor diseases into innovative clinical trials. A major focus of this work will be the investigation of the biology of normal and malignant stem cells and the translation of this knowledge into novel therapeutic approaches. New links to clinical hematology are expected to achieve higher standards of quality in clinical research and patient care. The new position will be able to use the structures which the NCT has created for conducting interdisciplinary treatment programs and effective clinical trials with special emphasis on investigator-initiated trials.

The successful candidate will have a record of outstanding productivity in the research field of stem cell biology and will not only be a competent and experienced physician and specialist in hematology/oncology who is thoroughly familiar with current procedures for the treatment of tumors and leukemia but will also be able to carry the responsibility for the organisation and supervision of high-standard clinical trials. Excellent clinical and pre-clinical research work including the use of pre-clinical animal models will be expected and the successful candidate will perform the duties of a senior physician entrusted with the care of cancer patients in the NCT hospital facilities.

The successful candidate will have a "habilitation" or comparable scientific qualifications and be a capable and experienced educator.

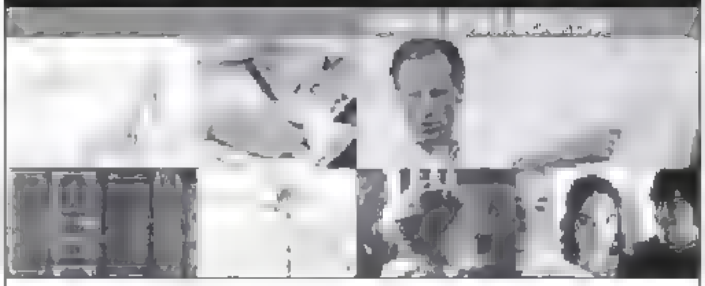
The position has been instituted as a permanent professorship, but in accordance with employment regulations (§ 50 Abs. I LHG) the contract for the first appointment as a professor must be limited. Exceptions can be made; especially in the case of applicants from foreign countries or non-academic institutions who otherwise would not be willing to accept this position. The contract can be renewed and converted into a permanent status without repeating the entire appointment procedure.

The German Cancer Research Center and Heidelberg University are committed to increase the representation of women in research work and encourage applications from qualified female scientists and physicians. Persons with disabilities will be given preference among equally qualified candidates.

Please send a letter of application, including a statement of current and future research interests, curriculum vitae, and a list of publications to the following address by January 31, 2008: Prof. Dr. Otmar D. Wiestler, Chairman of the Management Board, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg

W121525R

UNIVERSITY OF SOUTHERN DENMARK



- **Assistant professor in bioimaging in relation to renal and cardiovascular physiology and pathophysiology**

SDU - Odense

The Department of Physiology and Pharmacology, Institute of Medical Biology, Faculty of Health Sciences, University of Southern Denmark invites applications for a tenure-track position as assistant professor

Further information can be obtained from Head of Research, Prof. Boye L. Jensen, MD, PhD, Institute of Medical Biology, Telephone +45 6550 3796, E-mail: bljensen@health.sdu.dk or Prof. Ole Skøtt MD, DMSc, Head of Institute of Medical Biology, +45 6550 3752 e-mail oskott@health.sdu.dk

Full job description: www.jobs.sdu.dk and http://www.jobs.sdu.dk/vis_stilling.php?id=3901&lang=eng

DEADLINE FOR APPLICATIONS: January 31st, 2008 at 12:00 hrs.

www.sdu.dk vacant positions no. 071064

W121378R

SYDDANSKUNIVERSITET.DK

CNG

EU PROJECT MANAGER

Attached to the CEA/Institute of Genomics, the Centre National de Génotypage (CNG) is located on the principal campus for genomic research in France (Paris region), and is one of the leading European research centres using large-scale genetic approaches to investigate the molecular basis of human disease. CNG conducts major research programmes in diverse disease areas.

In the context of its FP7 European programmes, CNG will be coordinating a large-scale integrating project, which aims to revolutionise nucleic acid analysis methods.

We are seeking an experienced candidate to take charge of EU project management activities for this 4 year project (scientific work programme, administrative and financial coordination of the consortium, organising meetings and training courses, project website...). Ideally, you will have a PhD or Masters degree in genetics (or related subject) plus management training, and at least 2 years' experience of managing a European project. Excellent command of English required and, if possible, some knowledge of French. For full details of the position: http://www.cng.fr

To apply, please send CV, cover letter and names of three referees to: ProjectMgr@cng.fr (postal address: Human Resources, Centre National de Génotypage, CEA – Institut de Génomique, 2 rue Gaston Crémieux, 91057 Evry, France), quoting job ref. 'ProjectMgr', in subject heading of email, or envelope.

Closing date for applications: 31 January 2008

W121694R

BBSRC-funded CASE Studentships with AstraZeneca

Available in October 2008.

[illegible] one of the world's leading pharmaceutical companies, AstraZeneca is focused on turning good ideas into innovative, effective medicines that make a real difference in important areas of healthcare. As part of our long-term commitment to the discovery of medicines, applications are invited for the following PhD studentships between leading academic institutes and AstraZeneca commencing October 2008. Projects will be based in the academic groups and include a minimum three month placement at AstraZeneca, Alderley Park, Cheshire.

You should have, or expect to obtain, a first or upper second class honours degree in a relevant subject and meet the eligibility criteria set out in the BBSRC guidelines. Funding is for four years at the standard BBSRC rates with an additional contribution from AstraZeneca.

[illegible] stem cells in osteoarthritis

Prof. Ann Canfield (ann.canfield@manchester.ac.uk)
[illegible] Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester

To determine the functional role of microRNA in [illegible] therapeutic application

Dr Chris Murphy (c.murphy@imperial.ac.uk)
[illegible] Faculty of Pharmacology, Imperial College [illegible]

[illegible] respiration

Dr Jo Nash (josephine.nash@manchester.ac.uk)
[illegible] Imaging Science and Biomedical Engineering (ISBE), Faculty of [illegible], University of Manchester

Prof. Geoff Parker (geoff.parker@manchester.ac.uk)
[illegible] Imaging Science and Biomedical Engineering (ISBE), Faculty of [illegible], University of Manchester

[illegible] human type II topoisomerases

Prof. Caroline Austin (caroline.austin@ncl.ac.uk)
[illegible] Institute for Cell and Molecular Biosciences, University of Newcastle-upon-Tyne

Dr Micheala Frye (michaela.frye@cancer.org.uk)
[illegible] Cancer Research UK, Wellcome Trust Centre for Stem Cell Research, University of Cambridge

Dr Nina Balthasar (nina.balthasar@bristol.ac.uk)
[illegible] Department of Physiology, Faculty of Medical Sciences, University of Bristol

Prof. N G Morgan (noel.morgan@pms.ac.uk)
[illegible] Peninsula Pharmacology Unit, Peninsula Medical School, Universities of Exeter and Plymouth

[illegible] animal models

[illegible] Faculty of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast

[illegible] To apply for any of these studentships, your CV and names of two academic referees should be sent by email before [illegible] 2008 to the academic supervisor listed above.

careers.astrazeneca.co.uk

AstraZeneca
life inspiring ideas

SCIENCE OFFICER
FORESTS, THEIR PRODUCTS AND SERVICES (FPS)

The European Science Foundation provides administrative and scientific management for COST, its Domain Committees and its Actions through the COST Office in Brussels and corporate support services in Strasbourg. This is achieved through a support grant provided to ESF by the European Commission. COST is an inter-governmental system supporting European research networking. The ESF seeks a full-time Science Officer in the Domain of FPS for its COST Office in Brussels. The position will imply the management of the FPS Domain by catalysing and supporting researchers, Domain Committees and COST Actions, promoting inter-disciplinary research collaboration in a multi-disciplinary environment across Europe and beyond.

Position responsibilities:

- Implementing and supporting the scientific networking programme scheme, and approved programmes for the FPS Domain, under the direction of the Senior Science Officer Life Sciences;
- Delivering and advancing specific and quality papers and reports, related to the FPS Domain;
- Organising scientific quality control and responding to the COST Open Call related to the FPS Domain;
- Taking responsibility for the management of the Domain budgets;
- Providing scientific secretariat for the FPS Domain Committee and its Chair;
- Liaising with COST Domain Committee Chairs and other external scientific bodies and networks and providing support;
- Contributing to the Life Sciences Cluster activities;
- Promoting and centralizing publication needs according to the Domain requirements;
- Representing the COST Office in external meetings.

Profile and competencies:

Specific competencies

- Ph.D. or equivalent research experience in a field relevant to forestry economics (incl. pulp & paper) preferably with a further 5-10 years research experience in a relevant science area;
- Proven experience with grant assessment and review processes and experience of science management;
- Knowledge of European and national research structures and institutions and European and international science policy;
- Networking skills;
- High standard of spoken and written English, with a working knowledge of another European language being an advantage but not a requirement;
- Good working knowledge of MS Office systems and of electronic databases and Web sites Inter-personal competencies;
- Action-orientated, responsible and self-managed, creative and willing to take initiatives, and continuous improvement minded;
- Strong inter-personal and excellent communication and presentation skills within a multi-national context, including discretion, diplomacy and tolerance;
- Assertive and capability to guide decision-making procedures and to represent COST in the scientific community;
- Commitment to deliver on allocated tasks and respond in a timely manner to deadlines;
- Transparency in working and a team-orientated work ethic;
- Willingness to travel extensively.

We offer:

- An international working environment located in Brussels 1050, avenue Louise 149;
- Fixed duration contract of 4 years, with an initial probation period of 6 months;
- Competitive salary;
- Complementary insurances.

The selected person should preferably be available by 1 May 2009.

Electronic applications (motivation letter + CV) should be addressed to the COST Office Director and sent to job@cost.esf.org Reference code: SO FPS.

Interviews will be planned early February 2009.

For general details see http://www.cost.esf.org

Closing date: Wednesday 16 January 2009

SCIENCE OFFICER
MATERIALS, PHYSICAL AND NANOSCIENCES (MPNS)

The European Science Foundation provides administrative and scientific management for COST, its Domain Committees and its Actions through the COST Office in Brussels and corporate support services in Strasbourg. This is achieved through a support grant provided to ESF by the European Commission. COST is an inter-governmental system supporting European research networking. The ESF seeks a full-time Science Officer in the Domain of MPNS for its COST Office in Brussels. The position will imply the management of the MPNS Domain, by catalysing and supporting researchers, Domain Committees and COST Actions, promoting inter-disciplinary research collaboration in a multi-disciplinary environment across Europe and beyond.

Position responsibilities:

- Implementing and supporting the scientific networking programme scheme, and approved programmes for the MPNS Domain, under the direction of the Senior Science Officer Natural Sciences;
- Delivering and advancing specific and quality papers and reports, related to the MPNS Domain;
- Organising scientific quality control and responding to the COST Open Call related to the MPNS Domain;
- Taking responsibility for the management of the Domain budgets;
- Providing scientific secretariat for the MPNS Domain Committee and its Chair;
- Liaising with COST Domain Committee Chairs and other external scientific bodies and networks and providing support;
- Contributing to the Natural Sciences Cluster activities;
- Promoting and centralizing publication needs according to the Domain requirements;
- Representing the COST Office in external meetings.

Profile and competencies:

Specific competencies

- Ph.D. or equivalent research experience in a field related to materials, physics and nanosciences, preferably with a further 5-10 years research experience in a relevant science area;
- Proven experience with grant assessment and review processes and experience of science management;
- Knowledge of European and national research structures and institutions and European and international science policy;
- Networking skills;
- High standard of spoken and written English, with a working knowledge of another European language being an advantage but not a requirement;
- Good working knowledge of MS Office systems and of electronic databases and Web sites Inter-personal competencies;
- Action-orientated, responsible and self-managed, creative and willing to take initiatives, and continuous improvement minded;
- Strong inter-personal and excellent communication and presentation skills within a multi-national context, including discretion, diplomacy and tolerance;
- Assertive and capability to guide decision-making procedures and to represent COST in the scientific community;
- Commitment to deliver on allocated tasks and respond in a timely manner to deadlines;
- Transparency in working and a team-orientated work ethic;
- Willingness to travel extensively.

We offer:

- An international working environment located in Brussels 1050, avenue Louise 149;
- Fixed duration contract of 4 years, with an initial probation period of 6 months;
- Competitive salary;
- Complementary insurances.

The selected person should ideally be available by 1 April 2009.

Electronic applications (motivation letter + CV) should be addressed to the COST Office Director and sent to job@cost.esf.org Reference code: SO MPNS.

Interviews will be planned by the end of January 2009.

For general details see http://www.cost.esf.org

Closing date: Wednesday 16 January 2009

SCIENCE OFFICER
INDIVIDUALS, SOCIETIES, CULTURES AND HEALTH (ISCH)

The European Science Foundation provides administrative and scientific management for COST, its Domain Committees and its Actions through the COST Office in Brussels and corporate support services in Strasbourg. This is achieved through a support grant provided to ESF by the European Commission. COST is an inter-governmental system supporting European research networking. The ESF seeks a full-time Science Officer in the Domain of ISCH for its COST Office in Brussels. The position will imply the management of the ISCH Domain, by catalysing and supporting researchers, Domain Committees and COST Actions, promoting inter-disciplinary research collaboration in a multi-disciplinary environment across Europe and beyond.

Position responsibilities:

- Implementing and supporting the scientific networking programme scheme, and approved programmes for the ISCH Domain, under the direction of the Senior Science Officer Science in Society;
- Delivering and advancing specific and quality papers and reports, related to the ISCH Domain;
- Organising scientific quality control and responding to the COST Open Call related to the ISCH Domain;
- Taking responsibility for the management of the Domain budgets;
- Providing scientific secretariat for the ISCH Domain Committee and its Chair;
- Liaising with COST Domain Committee Chairs and other external scientific bodies and networks and providing support;
- Contributing to the Science in Society Cluster activities;
- Promoting and centralizing publication needs according to the Domain requirements;
- Representing the COST Office in external meetings.

Profile and competencies:

Specific competencies

- Ph.D. or equivalent research experience in the field of social sciences (in particular European politics sciences) preferably with a further 5-10 years research experience in a relevant science area with an emphasis on politics, sciences;
- Proven experience with grant assessment and review processes and experience of science management;
- Knowledge of European and national research structures and institutions and European and international science policy;
- Networking skills;
- High standard of spoken and written English with a working knowledge of another European language being an advantage but not a requirement;
- Good working knowledge of MS Office systems and of electronic databases and Web sites Inter-personal competencies;
- Action-orientated, responsible and self-managed, creative and willing to take initiatives, and continuous improvement minded;
- Strong inter-personal and excellent communication and presentation skills within a multi-national context, including discretion, diplomacy and tolerance;
- Assertive and capability to guide decision-making procedures and to represent COST in the scientific community;
- Commitment to deliver on allocated tasks and respond in a timely manner to deadlines;
- Transparency in working and a team-orientated work ethic;
- Willingness to travel extensively.

We offer:

- An international working environment located in Brussels 1050, avenue Louise 149;
- Fixed duration contract of 4 years, with an initial probation period of 6 months;
- Competitive salary;
- Complementary insurances.

The selected person should ideally be available by 1 April 2009.

Electronic applications (motivation letter + CV) should be addressed to the COST Office Director and sent to job@cost.esf.org Reference code: SO ISCH.

Interviews will be planned by the end of January 2009.

For general details see http://www.cost.esf.org

Closing date: Wednesday 16 January 2009

SCIENCE OFFICER
FOOD AND AGRICULTURE (FA)

The European Science Foundation provides administrative and scientific management for COST, its Domain Committees and its Actions through the COST Office in Brussels and corporate support services in Strasbourg. This is achieved through a support grant provided to ESF by the European Commission. COST is an inter-governmental system supporting European research networking. The ESF seeks a full-time Science Officer in the Domain of FA for its COST Office in Brussels. The position will imply the management of the FA Domain, by catalysing and supporting researchers, Domain Committees and COST Actions, promoting inter-disciplinary research collaboration in a multi-disciplinary environment across Europe and beyond.

Position responsibilities:

- Implementing and supporting the scientific networking programme scheme, and approved programmes for the FA Domain, under the direction of the Senior Science Officer Life Sciences;
- Delivering and advancing specific and quality papers and reports, related to the FA Domain;
- Organising scientific quality control and responding to the COST Open Call related to the FA Domain;
- Taking responsibility for the management of the Domain budgets;
- Providing scientific secretariat for the FA Domain Committee and its Chair;
- Liaising with COST Domain Committee Chairs and other external scientific bodies and networks and providing support;
- Contributing to the Life Sciences Cluster activities;
- Promoting and centralizing publication needs according to the Domain requirements;
- Representing the COST Office in external meetings.

Profile and competencies:

Specific competencies

- Ph.D. or equivalent research experience in a field relevant to food systems (incl. agricultural biotechnology), preferably with a further 5-10 years research experience in a relevant science area, with an emphasis on food systems;
- Proven experience with grant assessment and review processes and experience of science management;
- Knowledge of European and national research structures and institutions and European and international science policy;
- Networking skills;
- High standard of spoken and written English, with a working knowledge of another European language being an advantage but not a requirement;
- Good working knowledge of MS Office systems and of electronic databases and Web sites Inter-personal competencies;
- Action-orientated, responsible and self-managed, creative and willing to take initiatives, and continuous improvement minded;
- Strong inter-personal and excellent communication and presentation skills within a multi-national context, including discretion, diplomacy and tolerance;
- Assertive and capability to guide decision-making procedures and to represent COST in the scientific community;
- Commitment to deliver on allocated tasks and respond in a timely manner to deadlines;
- Transparency in working and a team-orientated work ethic;
- Willingness to travel extensively.

We offer:

- An international working environment located in Brussels 1050, avenue Louise 149;
- Fixed duration contract of 4 years, with an initial probation period of 6 months;
- Competitive salary;
- Complementary insurances.

The selected person should ideally be available by 1 May 2009.

Electronic applications (motivation letter + CV) should be addressed to the COST Office Director and sent to job@cost.esf.org Reference code: SO FA.

Interviews will be planned by the end of January 2009.

For general details see http://www.cost.esf.org

Closing date: Wednesday 16 January 2009

W121694R

Obra Social
Fundación "la Caixa"

"la Caixa"/CNIO
INTERNATIONAL PhD PROGRAMME
CALL FOR APPLICATIONS
Spanish National Cancer Research Centre (CNIO)

cnio
Centro Nacional de Investigaciones Oncológicas

During 2008, the Fundacion "la Caixa" will provide support for ten highly qualified graduate students to carry out their experimental work towards obtaining a PhD degree at the Centro Nacional de Investigaciones Oncológicas (CNIO) within a new International PhD Programme. "La Caixa" is the third largest Spanish financial institution. The CNIO maintains an outstanding scientific reputation based on the quality of its research programmes, the excellence of its publication record and its role in promoting scientific interaction and collaborations.

The CNIO, located in Madrid, Spain, offers interdisciplinary training and research opportunities to outstanding young graduates in biomedical sciences wishing to pursue an ambitious PhD project within an international scientific environment. The CNIO is one of the few European Cancer Research Centres of excellence that effectively combines basic and applied research. Currently, our Centre houses 25 basic and translational research groups supported by nine core units organised into five research programmes with extensive cross collaboration and interaction. The research programmes include Molecular Oncology, Cancer Cell Biology, Structural Biology and Biocomputing, Molecular Pathology and Human Cancer Genetics (for details of research activities see the CNIO's Scientific Report at http://www.cnio.es/ing/sr/index.asp). The CNIO also has a programme on Experimental Therapeutics exclusively dedicated to drug discovery efforts and hence, with limited access to graduate students.

In the context of this new **"la Caixa"/CNIO International PhD Programme**, we are seeking highly motivated PhD students interested in receiving ample cross-disciplinary training in state-of-the-art basic and applied cancer research. Students of any nationality holding a university degree (BSc, MSc, Diploma, DEA, Licenciatura, Laurea or equivalent), or who expect to be awarded with such qualification during the first half of 2008, are eligible to apply to the Programme. Candidates should have an excellent academic record, fluid knowledge of English and previous research experience during their undergraduate period. Co-authorship of publications in indexed journals will be positively considered.

The fellowships will be awarded on a competitive basis and will be renewable annually for up to four years. During the first two years, successful candidates will receive a total annual stipend of €18,550 plus a one time payment of €1,500 to cover miscellaneous expenses such as university fees. During the third and fourth year, the graduate students will receive a contract with a gross annual salary of €26,700 plus a one time payment of €1,700. In addition, they will receive full health and occupational insurance benefits. Short-listed candidates will be invited to visit the CNIO for an interview.

Application Deadlines: *First Call:* March 31st, 2008 *Second Call (if positions remain available):* June 30th 2008

All candidates must be selected by the end of July 2008.

For further information and to apply visit www.cnio.es/PhD ***or contact the Training Office at*** PhD@cnio.es

Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro, 3- 28029 Madrid-SPAIN

Tel +34 91 2246900 Fax. +34 91 2246980 Web www.cnio.es

W121741RM

HOKKAIDO UNIVERSITY FOUNDED 1876

Invitation of Applications for Tenure-Track Positions at Hokkaido University 2008 (Sapporo, Hokkaido, Japan)

Under the "Hokkaido University Leader Development System in the Basic Interdisciplinary Research Areas" project, we are inviting applications for Project Assistant Professors in 2008 tenure-track positions. We are recruiting young researchers who have the necessary qualities to become leaders of future basic interdisciplinary research areas. Both strong research abilities and comprehensive leadership abilities (planning skills, organizational skills, motivation and analytical skills and internationality) will be valued during personnel selection. Tenure-track appointees will be provided with a laboratory and will be obliged to actively participate in leader development programs.

1. No. of positions: Four Tenure-Track Project Assistant Professors (affiliated with the Hokkaido University Creative Research Initiative "Sousei") in basic interdisciplinary research areas.
The specific research fields covered by this recruitment are outlined on the homepage of the "Leader Development Station" (L Station), which is the tenure-track supporting organization.
http://www.cris.hokudai.ac.jp/l-station/

2. Terms of Employment: (1) Fixed-term employment as Project Assistant Professor until March 31, 2013.
(2) A mid-term evaluation will be conducted in fiscal 2011 to determine whether appointees can continue in the tenure-track positions. A final evaluation will be performed at the end of the term to determine whether the appointees will be hired in tenure positions within the Hokkaido University hosting section.

3. Qualifications: Candidates should hold PhD degrees (or corresponding degrees gained overseas).

4. Research environment: The following research expenses are scheduled to be provided. Five million yen as startup expenses in the first fiscal year; three million yen in the second fiscal year; two million yen in the third fiscal year; and 1 million yen each in the fourth and fifth fiscal years. Approximately 50 m² of research space in the Creative Research Initiative "Sousei" will be also provided. The open facility will also be available.
* For more information about the open facility, see the following Website. http://www.cris.hokudai.ac.jp/openfacility/

5. Application deadline: Received on or before February 4 (Monday), 2008.

6. Screening schedules: First screening: Late-February.
Second screening and final decision - scheduled for late March.

7. Starting date: As soon as possible in or after April 2008.

8. Where to submit application documents:
Leader Development Station "L Station"
Hokkaido University Creative Research Initiative "Sousei"
North 21, West 10, Kita-ku, Sapporo 001-0021

Write "Enc. Application Form for Tenure-Track Positions, Field No. " on the envelope and send it by registered mail.

Applications from female scientists are also encouraged.

9. For more information about research fields, please refer to the home page
http://www.cris.hokudai.ac.jp/l-station/

JP119035R

nature chemistry

Associate Editors

The Nature Publishing Group is pleased to announce the launch of *Nature Chemistry* in 2009. Following the success of *Nature Materials*, *Nature Chemical Biology and Nature Physics*, and given the strength of the parent journal *Nature*, we fully expect *Nature Chemistry* to seize the commanding heights of the chemistry-publishing landscape.

Alongside the highest-quality original research, *Nature Chemistry* will cover news, commentary and analysis from and for the chemistry community, as well as striving to develop a voice that chemists care about.

As part of this exciting new publishing venture, we are now seeking three Associate Editors for *Nature Chemistry*, to be based in our London, Boston and Tokyo offices.

Applicants should have a PhD in a chemistry-related discipline, with demonstrable research achievements. Although postdoctoral experience is preferred (not required), emphasis will be placed on broadly trained applicants with a good knowledge of the chemistry community. Key elements of the position include the selection of manuscripts for publication, and commissioning, editing and writing other content for the journal. Candidates who wish to be considered for the role in our Japan office must demonstrate a good understanding of the East Asian research communities (in particular Japan, China and Korea) as well as being fluent in English and preferably an Asian language (Japanese, Chinese or Korean).

These are demanding and extremely stimulating roles, which call for a keen interest in the practice and communication of science. The successful candidates will, therefore, be dynamic, motivated and outgoing, and must possess excellent interpersonal skills. The salary and benefits, will be competitive, reflecting the critical importance and responsibilities of each position.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (no more than 500 words) on a recent paper from the chemical literature, and a brief cover letter explaining their interest in the post, salary expectations, and indicating whether they wish to be considered for a position in London, Boston or Tokyo.

To apply please send your CV and covering letter, quoting reference number **NPG/LON/797** to Denise Pitter at londonrecruitment@macmillan.co.uk

The closing date for applications is Thursday 31st January 2008.

nature publishing group npg

IN121118R

---

nature

Head of Community Business Development

During the last decade, scientific communication has undergone a revolution – most research information is now accessed online rather than in print. Nature.com is already one of the foremost scientific destinations on the web, with about two million registered users, and several million visitors and many tens of millions of page views each month. As well delivering the unsurpassed scientific content for which the company is renowned, NPG's web presence also provides popular email and RSS alerting services, as well as a range of innovative and award-winning participative features. We are now seeking a commercially, technologically and scientifically astute individual to oversee the further development and integration of these services in order to maximize and realise their business potential. This person will play a central role in NPG's evolution as a scientific communication company. They will be based in London or New York and will report to the Publishing Director, Nature.com.

This role will focus on using online approaches to develop a better understanding of, and deeper relationships with, each of our users. By serving them better we intend ultimately to attract attention and usage from all professional scientists, and by using these services as the foundation for new businesses we intend to continue NPG's rapid evolution as an online scientific communication company. This role will involve line management responsibility for our existing social software teams, as well as the appointment of further staff in the areas of online marketing and web statistics.

We are seeking someone with a clear strategic sense of how the web is evolving, sufficient technical knowledge to work closely with software developers, a clear strategic vision for the future of communities on Nature.com, and experience in developing, promoting and running successful participative websites. A demonstrable interest in science is essential, an educational background in science would be preferable.

To apply, please send your CV and covering letter (quoting reference NPG/LON/774), stating your current salary to Kerrie Willsher, Personnel, Macmillan Publishers at londonpersonnel@macmillan.co.uk

Closing Date: 31st January 2008.

nature publishing group npg

IN120807R

The CANARY ISLANDS OCEANIC PLATFORM (PLOCAN) seeks a

SENIOR MANAGER FOR DIRECTOR

The CANARY ISLANDS OCEANIC PLATFORM (PLOCAN) is a new facility for oceanic research Science, created as a Consortium of the Spanish Ministry of Education and Science and the Canary Islands Government, as part of the implementation of the Spanish Research Infrastructures Roadmap.

The Platform is located offshore, at the continental platform border in the proximity of Gran Canaria Island, where the Consortium headquarters is located.

The Director reports to the Governing Council and is responsible for managing the Platform construction and operations, and for maximising its readiness and effectiveness for scientific research. The Director is responsible for recruiting and maintaining high quality scientific, technical and administrative staff, developing an annual budget for review and approval, and proposing the short-and long-range plans for the Platform.

Salary range and starting date are to be negotiated.

Review of applications will begin on February 2008, and the recruitment will remain open until the position is filled.

Additional information of characteristics and conditions of the position on **www.plocan.eu**

Gobierno de Canarias FEDER

W121738R

UNIVERSITÄT ZU KÖLN

The Institute of Genetics at the Faculty of Mathematics and Natural Sciences of the University of Cologne invites applications for the position of a tenured appointment as

Professor (W3) in Genetics

We seek individuals with a proven track record of research and international recognition in Genetics, particularly with respect to the biology of aging, sustained external funding, and teaching experience. The successful candidate is expected to complement the ongoing life science research programmes in Cologne such as the Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), as well as collaborative research centres (SFB 572, 635, 670, 680) and to participate in the teaching programmes of the Bio-Centre Cologne.

The University of Cologne is an equal opportunity employer in compliance with German disability laws. Applications from women and disabled persons are particularly welcome.

Applicants are requested to send a full curriculum vitae, a complete list of publications, a summary of current and future research interests, an outline of teaching experiences and collaborations, a list of external funding, copies of degree documents, and a selection of maximally five reprints **by January 18, 2008** to the **Dean of the Faculty of Mathematics and Natural Sciences, University of Cologne, Albertus-Magnus-Platz, D-50923 Cologne, Germany.**

W121477R

---

www.naturejobs.com

ITT DUBLIN

NUI MAYNOOTH

DCU

Centre for Applied Science for Health

POSTDOCTORAL FELLOWSHIPS AT ITT DUBLIN

Starting salary: €39,682 p.a.

1. INVESTIGATION OF HOST BACTERIUM INTERACTIONS IN THE LUNG (Code: ITT HEA PD01)
   For information, contact: Dr Siobhan McClean – siobhan.mcclean@ittdublin.ie
   or Dr. Máire Callaghan – maire.callaghan@ittdublin.ie
2. ELECTROCHEMICAL SENSORS FOR NEUROCHEMICAL DETECTION (Code: ITT HEA PD02)
   For information, contact: Dr. Eithne Dempsey – eithne.dempsey@ittdublin.ie
   or Dr. Tim McCormac – Tim.mccormac@ittdublin.ie

RESEARCH ASSISTANTS BASED AT ITT DUBLIN

Fixed term 24 month contracts. Salary scale: €23,000 to €34,000

1. Screening of compounds for antimicrobial effect, anti-cancer activity, and for cytotoxicity (Code: ITT HEA RA1)
   For information, contact: Dr. Siobhán McClean – siobhan.mcclean@ittdublin
   or Dr. Bernie Creaven – Bernie.creaven@ittdublin.ie
2. Scale-up and Characterisation of Antimicrobial Peptides Produced by Staphylococcus sp. (Code: ITT HEA RA2)
   For information, contact: Mr. John Behan – John.behan@ittdublin.ie
   or Dr. Mary Costello – mary.costello@ittdublin.ie

POSTGRADUATE RESEARCH STUDENTSHIPS BASED AT ITT DUBLIN

Postgraduate students based at ITT Dublin register initially for a M.Sc. Funding is available for three years to facilitate transfer to the PhD Register subject to satisfactory performance in the first 18 months of the project. Annual stipend €16,000

1. Masters In Engineering – Biomedical Engineering (Code: ITT HEA ME1)
   For information contact: linda.hickey@ittdublin.ie or gerard.ryder@ittdublin.ie
2. M.Sc./ Ph.D. in Chemistry
   a. Synthesis of Bacterial Biofilm Inhibitors (Code: ITT Dublin HEA PG1)
   For information, contact: Dr Fintan Kelleher – fintan.kelleher@ittdublin.ie
   or Dr. Adrienne Fleming – Adrienne.fleming@ittdublin.ie
   b. Design and Synthesis of Coumarin-Based Agents As Potential Treatments For Hospital Acquired Infections (Code: ITT Dublin HEA PG2)
   For information, contact: Dr Maureen Walsh – Maureen.walsh@ittdublin
   or Dr Bernie Creaven – Bernie.creaven@ittdublin.ie
3. M.Sc./ Ph.D. in Biology
   a. Role of Growth Factors in Mediating Metastatic Cancer Following Standard Anti-Cancer Chemotherapy. (Code: ITT HEA PG2, based at ITT Dublin)
   For information, contact: Dr Denise Egan – denise.egan@ittdublin.ie
   b. M.Sc. – Antiplatelet Therapy, Adenosine Metabolism and Stroke (Code: Code: ITT HEA MS2, Stipend €10,800 p.a. for 21 months.
   For information, contact: Dr Denise Egan – denise.egan@ittdublin.ie
   or Dr Dominick McCabe – Dominick.mccabe@amnch.ie

To apply for any of the above positions at ITT Dublin, send a completed application by email to: human.resources@ittdublin.ie or post to HR office, ITT Dublin, Old Blessington Road, Dublin 24, Ireland. Closing date for receipt of all applications is 17:00 p.m on January 28th 2008

POSTGRADUATE RESEARCH STUDENTSHIPS AT AMNCH

Successful candidates for positions at AMNCH will be expected to register for a PhD at Trinity College [redacted]

1. Ph.D. in Biology (annual stipend: €16,000; based in AMNCH)
   a. Investigation Of Mechanisms Of Pathogenesis In Irritable Bowel Syndrome (Code: AMNCH PG1)
   For information, contact: Dr. Maria O Sullivan (AMNCH) – maria.osullivan@tcd.ie
   or Dr. Siobhán McClean (ITT Dublin) – siobhan.mcclean@ittdublin.ie
   b. Anti-Infective strategies in biofilm related infection (Code: AMNCH PG2)
   For information, contact: Prof. Philip Murphy – Philip.murphy@amnch.ie

To apply for any of the above positions at AMNCH, send a detailed CV by email to the relevant contact person(s). Closing date for receipt of all applications is 17:00 p.m on January 28th 2008.

POSTGRADUATE RESEARCH STUDENTSHIPS AT NUI MAYNOOTH – Department of Chemistry

(Annual stipend: €16,000)

1. Ph.D. in Chemistry
   a. Synthesis, Characterisation and Physiochemical Properties of Novel Quinolone Derivatives (Code: NUIM PG1) For information, contact: Dr. John Stephens – john.stephens@nuim.ie
   b. Design, Development And Characterisation Of A Nav Biosensors Systems For In-Vivo Neurochemical Monitoring Of Energy Metabolites (Code: NUIM PG2)
   For information, contact: Prof. John P. Lowry – John.Lowry@nuim.ie

To apply for any of the above positions at NUI Maynooth, send a detailed CV by email to the relevant contact person(s). Closing date for receipt of all applications is 17.00 p.m on January 28th 2008.

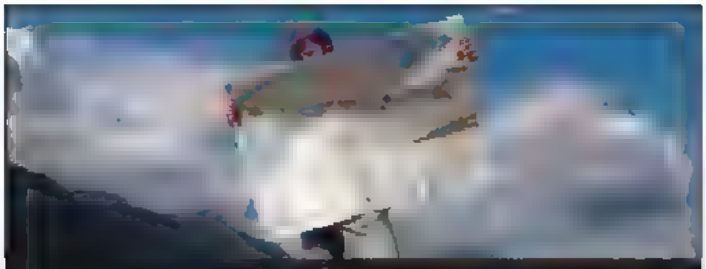
Please note that applicants who are selected for interview may be required to travel to Dublin at their own expense. ITT Dublin is an Equal Opportunities Employer

Higher Education Authority
An Údarás um Ard-Oideachas

NDP
NATIONAL DEVELOPMENT PLAN

European Union
Structural Funds

W-12-1542R



Lost in today's ever-changing biosciences environment?

...get your bearings with these Stanford School of Medicine Career Center (SoMCC) seminars presented by *Naturejobs*.

Naturejobs and the Stanford School of Medicine have collaborated to bring you this video series featuring SoMCC "Industry Insights" and "Careers in Science" programs. This monthly series, delivered by top experts within the biomedical sciences and healthcare industries, will allow you to:

- OBTAIN OF THE LATEST TRENDS AND FORCES SHAPING THE BIOSCIENCES
- GAIN VISIBILITY INTO THE DIVERSE SETTINGS WHERE BIOMEDICAL PROFESSIONALS ENGAGE
- LEARN FROM FIRST-HAND PERSPECTIVES OF THE FOREMOST LEADERS IN BUSINESS AND ACADEMIA

Visit www.naturejobs.com/magazine/video to stream or download the following presentations:

- *Convergence of Science, Banking, and Finance with MDS Capital*, Nandini Tandon, Ph.D, MDS Capital
- *How Should We Be Developing Drugs in the 21st Century?*, Hal Barron, MD, Genentech

And stay tuned for these seminars coming soon:

Intellectual Property Management & Technology Transfer, Panel of Experts
Science & the Media, Donald Kennedy, PhD, Emeritus Professor, Stanford
The Future of Personalized Medicine, with Agilent Technologies

If you are interested to learn more about the SoMCC, please contact Suzanne Frasca, Program Coordinator, at (650) 725-7887 or somcareers@stanford.edu.

naturejobs

nature jobs
making science work

Head of Classified Advertising

Classified advertising – in scientific jobs, events and other areas – is an established part of Nature Publishing Group's business, but with very substantial growth potential. In particular, the migration of jobseekers to the web creates opportunities to exploit our established track record in online innovation, serving customers in novel ways and creating new business opportunities. We are now seeking a person to lead these developments. This position will be based in New York or London, with regular international travel required, and will report to the Publishing Director, Nature.com.

The role will encompass line management of sales, editorial and technical colleagues, as well as providing strategic direction for the business. Some specific responsibilities include management of the established sales team in London and New York, development of compelling and profitable upsell offerings as part of Naturejobs' 'freemium' business model, develop new ways of engaging with jobseekers, event attendees and other important audiences, oversight of revenue and market-share growth in established markets such as the US and oversight of Naturejobs web operations.

We are therefore seeking a candidate with a proven ability in sales, a sound and up-to-date knowledge of online trends, a clear strategic vision for the business coupled with sound tactical judgement, a strong leadership style and substantial experience in classified advertising, especially in an online environment. An educational background in science or medicine is also preferable.

To apply, please send your CV and covering letter (quoting reference NPG/LON/771), stating your current salary to Kerrie Willsher, Personnel, Macmillan Publishers at londonpersonnel@macmillan.co.uk

Closing Date: 31st January 2008

nature publishing group

IN120808R

Technische Universität Berlin

unicat

The **Cluster of Excellence (CoE) "Unifying Concepts in Catalysis" in Berlin** seeks for 3 full professorships with tenure (W3):

Full Professorship "Biocatalysis" (TU Berlin)
Full Professorship "Functional Materials" (TU Berlin)
Full Professorship "Structural Biology/Biochemistry" (HU Berlin)

1) A Full Professorship in **Biocatalysis** (Salary / Bes.Gr. W3) is opened in the Institute of Chemistry, Faculty II - Mathematics and Sciences of the Technische Universität Berlin.

The successful candidate will take over teaching responsibilities within the Bachelor and Master programme in Chemistry, including courses in Molecular Biology and Bio-chemistry, and is expected to strengthen the field of "Biophysical and Biological Chemistry" as an optional part of the Master programme. Research activities should lie in a modern field of Biocatalysis such as structure-function analysis of metalloenzymes, combinatorial biosynthesis, or genome mining. Participation in the Cluster of Excellence **"Unifying Concepts in Catalysis"** is expected.

2) A Full Professorship in **Functional Materials** (Salary / Bes.Gr. W3) is opened in the Institute of Chemistry, Faculty II Mathematics and Sciences of the Technische Universität Berlin.

The successful candidate will take over teaching responsibilities within the Bachelor and Master programme in Chemistry and is expected to strengthen the fields of "Materials Chemistry" and "Catalysis and Synthesis" as optional parts of the Master programme. Research activities should lie in a modern field of Functional Materials such as nanostructured and mesostructured materials, hybrid materials, self assembled or biomimetic materials. Participation in the Cluster of Excellence **"Unifying Concepts in Catalysis"** is expected.

Candidates for 1) and 2) must fulfil the requirements for professorship appointments according to § 100 of the Berliner Hochschulgesetz (BerlHG), and should have teaching experience and an outstanding research record.

The Technische Universität Berlin envisages to ensure equal opportunity for men and women, applications from female candidates with the advertised qualifications are explicitly solicited. In case of qualifications of the same standards, seriously handicapped persons will be given preference.

Please send your written application with no. of advertisement within 4 weeks to the following address:

Präsident der Technischen Universität Berlin
Fakultät II - Institut für Chemie - Sekr. TC 1
Strasse des 17 Juni 124
D - 10623 Berlin

3) The Faculty of Mathematics and Natural Sciences I, Institute of Biology at Humboldt-Universität zu Berlin (HU) invites applications from scientists with experience in research and teaching for a Full Professorship for **Structural Biology/Biochemistry** (Salary/ Bes.Gr. W3) at the earliest possible.

We will consider applicants with an established track record in any aspect of Structural Biology though preference may be given to applicants whose research complements existing research in the department and the Cluster of Excellence "Unifying Concepts in Catalysis". The Professor is expected to demonstrate a strong commitment to excellence in teaching 'Biochemistry in undergraduate and graduate programmes for biology, biophysics and catalysis students. The successful candidate is expected to participate in existing international research projects such as the "Integrative principles of Catalysis" and the Collaborative Research Centres (SFB 429 "Molecular Physiology, Energetics and Regulation of Primary Plant Metabolism", SFB 498 'Protein cofactor interactions' SFB 740 'From Molecules to Modules. Organisation and dynamics of functional units in cells') and the Interdisciplinary Centre for Biophysics and Bioinformatics at the Humboldt-Universität zu Berlin.

Applications should include a curriculum vitae, lists of publications and previously taught courses, a statement of research and teaching interests, and up to five selected publications and are to be sent with above reference number PR/027/07 within 4 weeks to:

Humboldt-Universität zu Berlin
Dekan der Mathematisch-Naturwissenschaftlichen Fakultät I
Prof. Dr. Christian Limberg
Unter den Linden 6
10099 Berlin

To accelerate the process, applicants are kindly requested to send their application materials both in written form as well as electronically via the internet. For further details, see https://www2.physik.hu-berlin.de/ss/struct-bio/. Application materials will not be returned. Therefore, you are requested to send only copies of all documents.

Applicants must meet the legal requirements for appointments of professors in accordance with § 100 of the "Berliner Hochschulgesetz". Habilitation or documented evidence of equivalent scientific qualifications is required.

HU is an equal opportunity employer committed to the advancement of individuals without regard to race, colour, religion, sex, age, national origin, ethnicity, disability or any other protected status. HU seeks to increase the proportion of female faculty members. Thus qualified women are particularly encouraged to apply.

W121745R

Visit
www.naturejobs.com
your source for
all career prospects
naturejobs
Making science work

UCL

The Eastman Dental Institute

Director of UCL Eastman Dental Institute Ref: EDI/08/01

The Faculty of Biomedical Sciences at UCL is one of the world's leading centres for biomedical research and teaching. The Dean, Professor Ed Byrne, seeks to appoint a new Director for the Eastman Dental Institute (EDI) to provide strategic leadership and help drive forward the Faculty's development plan.

The EDI is a postgraduate dental school with a renowned international profile. It is committed to innovative, high quality research, excellence in graduate teaching and exemplary patient care in close collaboration with UCLH NHS Foundation Trust (UCLH). The new Director will:

- Be a strategic and entrepreneurial leader with vision and the ability to influence, combined with strong communication and interpersonal skills
- Develop a strategy to ensure that EDI supports UCL's position at the forefront of international biomedical research and teaching
- Work with UCL and UCLH to ensure that the relocation of the Eastman to a new site supports this strategy, fostering strong relationships within the Faculty, UCL and UCLH
- Demonstrate experience of generating external income streams for teaching and research and be able to develop fruitful relationships with funding bodies, other organisations and the commercial sector
- Understand the complexities and issues associated with the provision of dental postgraduate education for the UK and overseas markets, and demonstrate experience in delivering and developing postgraduate education

Applications are invited from candidates with excellent management and leadership skills. A standing as an eminent, internationally-renowned researcher and/or educator would be an advantage.

The candidate brief and job description, including contact details for further information, can be downloaded from:

http://www.eastman.ucl.ac.uk/about/vacancies/index.html

Closing date: 29th February 2008

We particularly welcome applicants from an ethnic minority as they are currently under-represented within UCL at this level. This is in line with Section 38 of the Race Relations Act 1976.

eastman DENTAL INSTITUTE

J121403R

International Risk Governance Council

W121128R

Risk Research Associate

Based in Geneva, Switzerland, the International Risk Governance Council (www.irgc.org) seeks a research associate with a PhD and experience in risk assessment, risk management and risk regulation. The successful applicant will focus on supporting two IRGC projects. The first explores and explains deficits in global risk governance and links these deficits to major global risks. The second involves a review of different regulatory approaches and seeks to develop recommendations for alternative regulatory strategies for diverse risk types. In addition to demonstrable knowledge of the fields of work, we require a high standard of written English and the ability to work independently.

To apply for this position, please send a cover letter, résumé, writing samples and names of three references to:
Christopher Bunting
Secretary General
at **info@irgc.org**

irgc International risk governance council

Join WHO/TDR's innovative drug discovery networks

Seeking new medicinal chemistry centers in academia or industry.

Join a growing international North/South network for the discovery of new drug leads for infectious diseases of poverty. We are looking for investigators and institutions who can provide bench space and guide postdoctoral chemists in synthesizing compound analogues to explore structure activity relationsh ps and optimize anti-parasitic activity of available TDR coumpounds.

The fellows will be supported by TDR grants covering salaries and reagents initially for one year. Selected nstitutions or companies will work as part of the existing medicinal chemistry network and will nteract with the TDR compound evaluation and DMPK networks.

Applications from institutions and companies in develop ng countries are strongly encouraged.

Application deadline: **January 25, 2008**

For complete details and application, visit:
www.who.int/tdr/grants/grants/bi3_letter_interest.htm
or e-mail Dr Solomon Nwaka at: **nwakas@who.int**

W121378R

ACADEMY OF ATHENS

BIOMEDICAL RESEARCH FOUNDATION

The Biomedical Research Foundation of the Academy of Athens invites applications for a faculty position at the

ASSISTANT PROFESSOR LEVEL

in "Molecular Biology and Genetics of ageing using the model organism C. elegans", Center for Basic Research II, Division of Genetics and Gene Therapy.

Successful applicants should have a demonstrated track record in the use of the model organism C. elegans to study mechanisms of ageing.

Applications should include a curriculum vitae, a statement of research interests, the names and addresses of three references, and should be sent by 4th February, 2008 to:

Dimitris Thanos. Ph.D
Biomedical Research Foundation
Academy of Athens
4, Soranou Efesiou Street
Athens 11527, Greece
Tel: +30-210-6597244
Email: thanos@bioacademy.gr

W121631R

MRC Medical

MRC Epidemiology Unit, Cambridge

PhD Studentships 2008

The Medical Research Council Epidemiology Unit (www.mrc-epid.cam.ac.uk) invites applications for MRC funded PhD studentships in genetic epidemiology of diabetes and obesity, genetic and environmental determinants of infancy and childhood growth, physical activity epidemiology, dietary and nutritional epidemiology, and the development and evaluation of interventions to promote physical activity and to prevent obesity and diabetes, and their consequences.

The MRC PhD studentships are 3-year positions for those who already have a Masters in an appropriate scientific discipline. Opportunities exist for 4-year studentships for those wishing to undertake the MPhil in Epidemiology/Public Health course in the first year of their PhD.
The MRC PhD studentships include college and university fees and a stipend.

The academic requirement for entry is a first or upper second class degree or equivalent.

The MRC PhD studentships within this multi-disciplinary unit include diverse training opportunities for all aspects of research and encourage the development of both academic and generic research skills.

Graduate students register for their PhD with the University of Cambridge, belong to one of its Colleges, and are trained at the MRC Epidemiology Unit. Studentships will commence in October 2008.

Full funding for these positions is available to UK nationals and UK residents. Partial funding is available to applicants from the European Economic area (EEA). Please refer to the MRC Student eligibility criteria at: http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002632 for further information.

Potential PhD students should send a full CV, a covering letter and contact details of two referees to: Graduate Studies, MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital - Box 285, Hills Road, Cambridge CB2 0QQ, UK or email: Graduatestudies@mrc-epid.cam.ac.uk For enquiries about the studentships please email: Graduatestudies@mrc-epid.cam.ac.uk

Closing date: 11 February 2008.

Interview date: 29 February 2008.

For further information about the MRC visit www.mrc.ac.uk

The MRC is an Equal Opportunities Employer

'Leading science for better health'

L12 361R

---

nature nanotechnology

Associate Editor(s)

Nature Nanotechnology is a prestigious journal covering all areas of nanoscience and technology. We have exciting opportunities available for a chemist and a physicist/materials scientist to join our editorial team as Associate Editors.

These are demanding and intellectually stimulating positions. Applicants should have a PhD and preferably post-doctoral experience in an area of chemistry or physics/materials science related to nanoscience and technology. Broad scientific knowledge and good word skills are essential.

The successful candidates will work closely with the Editor on all aspects of the journal including manuscript selection, and commissioning, editing and writing other content for the journal and its website. Liaising with the international nanoscience and technology community is a central part of both jobs and the successfu candidates will be expected to attend conferences and visit laboratories around the world. The positions will be based in our London or Boston offices.

Applicants should send a covering letter (including their salary expectations), a CV, and a News & Views style piece (500 words or less) about a recent paper (or papers) in the literature to Denise Pitter, Personnel Assistant at London.recruitment@macmillan.co.uk Please quote reference number NPG/LON/788.

Closing date Thursday 31st January 2008.

nature publishing group npg

IN121125R

Career articles from Naturejobs

- **Naturejobs Prospect:** quick takes on career implications of current events
- **Naturejobs Special Report:** examinations of jobs issues on both sides of the bench
- **Naturejobs Careers & Recruitment:** discipline-by-discipline exploration of opportunities
- **Naturejobs Regions:** tours of scientific hubs
- **Naturejobs Movers:** traffic reports that follow high-profile scientific globe-trotters and sector-hoppers
- **Naturejobs Postdocs & Students:** guides to taking a step toward a permanent position

www.naturejobs.com

naturejobs
making science work

nature publishing group npg

Head of Methods, Protocols and Products

An area of particularly rapid recent growth for Nature Publishing Group (NPG) has come from the launches of two new titles, Nature Methods and Nature Protocols. NPG is now seeking a talented individual to lead them into their next phase, and to develop new businesses in the area scientific methods, protocols and products. This position will be based in either our New York or London office.

The role will involve publishing responsibility for Nature Methods and Nature Protocols – including the continued development of their innovative business models. It will also encompass the development of related products and services in order to meet the information and other needs of scientists who conduct laboratory experiments.

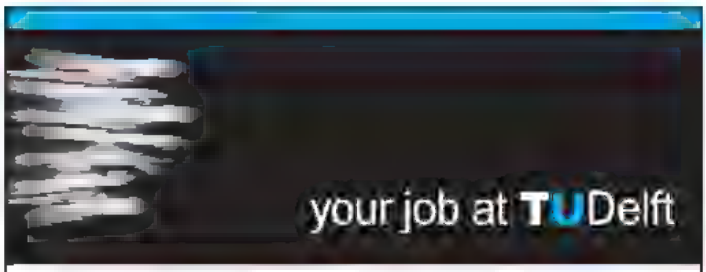
We are therefore seeking a candidate with a sound grasp of the ways in which scientists work, an up-to-date knowledge of online trends, a clear strategic vision for the business coupled with sound tactical judgment, excellent numerical and analytical skills and willingness, flexibility and energy in taking initiative and assuming responsibility. An educational background in science or medicine would be preferable.

To apply, please send your CV and covering letter (quoting reference NPG/LON/773), stating your current salary to Kerrie Willsher, Personnel, Macmillan Publishers at londonpersonnel@macmillan.co.uk

Closing Date. 31st January 2008

nature publishing group npg

IN120866R



CHALLENGE THE FUTURE

Assistant/Associate Professor of Physics Confocal Electron-Light Microscopy

Department:	**Imaging Science & Technology**
Level:	**PhD**
Working hours:	**Full-time**
Term of contract:	**Tenure-track or tenured as applicable**
Salary:	**Maximum of € 5670 per month gross with a full-time appointment**

The department is part of the Faculty of Applied Sciences. The professor should strengthen our research in imaging science and technology, specifically in instrumentation and applications for high resolution microscopy. The first challenge is to lead the development of a combined electron and light microscope, where lab-on-a-chip technology will be used to manipulate biological specimens in the sample chamber. We seek a physicist with proven skills in instrumentation and in networking with users who have a biology background. Other responsibilities will include teaching and coaching students.

Information and application

For more information about the position please visit:

www.jobsinDelft.nl

W121636R

TUDelft

University of Oxford

Department of Earth Sciences

Research Associates/Fellows in Organic Geochemistry and Cosmochemistry

The Department seeks to appoint an Organic Geochemist and a Cosmochemist to conduct and support research in geochemistry. The Department has a long standing reputation for excellence in geochemistry, and is expanding its research programs following the arrival of Professor Alex Halliday. The geochemistry research programs include various aspects of earth, environmental and planetary sciences and are supported by a wide range of analytical instrumentation that provides Oxford with one of the best-equipped facilities in the world. We are seeking talented and enthusiastic individuals who are keen to work in a dynamic, interactive and technically innovative geochemistry research facility. The Research Associates/Fellows in Organic Geochemistry and in Cosmochemistry will form a very important part of the new research team being assembled at Oxford.

Dependant on skills and qualifications, the successful candidates will be appointed either as a Research Associate (£26,666 - £32,796 p.a.), or a Research Fellow (£33,779 - £40,335 p.a.) The appointments will be for two years in the first instance.

Letters of application with a full CV and contact details of three referees (at least one of whom should be a current or previous employer) should be sent to Caroline Hutchings, Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR or E-mailed to Caroline.Hutchings@earth.ox.ac.uk and received no later than 17 January 2008, quoting reference number DG/07/013 for Organic Geochemistry and DG/07/014 for Cosmochemistry post.

Further Particulars are available from www.earth.ox.ac.uk/department/geochem.pdf

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U121066R

www.ox.ac.uk/jobs

The Gatsby Charitable Foundation

Sainsbury PhD Studentships in Plant Science

Enhanced postgraduate studentships, each for four years, starting in October 2008, will once again be awarded by the Gatsby Charitable Foundation, one of the Sainsbury Family Charitable Trusts. Each of the supervisors below will select a candidate who will then compete at interview, with Sainsbury Undergraduate students, for one of up to four Sainsbury PhD Studentships. Interviews will be held in London on Friday, 7 March 2008. It would be expected that the studentship holder spends six months during their 3rd or 4th year at another university/institute to gain additional experience.

Interested applicants should write, attaching a CV, before 25 January 2008, to one or more of the following.

Do plants survive stress by destroying RNAs?
Professor Brendan Davies, Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT
Email b.h.davies@leeds.ac.uk

Defining the role of the ABI4 transcription factor in the sugar regulated control of storage oil breakdown in *Arabidopsis*
Professor Ian Graham, Department of Biology (Area 7), University of York, PO Box 373, York YO10 5YW
Email ag1@york.ac.uk

Evolution of land-plant growth regulatory mechanisms
Professor Nicholas P Harberd, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB
Email nicholas.harberd@plants.ox.ac.uk

Calcium signalling controlling responses to stress
Professor Marc Knight, Institute of Plant and Microbial Sciences, School of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE
Email m.r.knight@durham.ac.uk

Defining the mechanisms of specificity in the symbiosis signalling pathway of legumes
Dr Giles Oldroyd, John Innes Centre, Norwich Research Park, Norwich NR4 7UH
Email giles.oldroyd@bbsrc.ac.uk

Investigating effector function and delivery in the rice blast fungus *Magnaporthe grisea*
Professor Nick Talbot, School of Biosciences, University of Exeter, Geoffrey Pope Building, Streatham Campus, Exeter EX4 4QD
Email n.j.talbot@exeter.ac.uk

U120891R

St. Jude Children's Research Hospital

FACULTY

Department of Structural Biology

St. Jude Children's Research Hospital (SJCRH), a premier center for biomedical investigation located in Memphis, Tennessee, USA, is seeking to fill two faculty positions in the Department of Structural Biology. Research in the Department is highly interactive within the hospital and centers on understanding the molecular basis of biological processes and human diseases. Examples of ongoing research include mechanistic studies of protein degradation, cell cycle regulation, tumor suppressor function, signal transduction, transcriptional regulation, DNA repair, structure-based drug design and lipid metabolism. A wide range of structural techniques are available in-house, including X-ray crystallography, NMR spectroscopy and high level computing. Regular access to synchrotron radiation is guaranteed through membership of SER-CAT at the Advanced Photon Source.

SJCRH is a hospital and basic research institute that focuses on the fundamental causes and treatment of catastrophic childhood diseases including cancer, infectious diseases and genetic disorders. Founded by Danny Thomas in 1962, the hospital currently includes some 150 basic and clinical investigators organized into a traditional academic environment. The research environment at SJCRH is highly interactive, with opportunities to collaborate with investigators in other Departments, including Biochemistry, Chemical Biology and Therapeutics, Developmental Neurobiology, Genetics and Tumor Cell Biology, Hematology, Oncology, Immunology, Infectious Diseases, Molecular Pharmacology, Pathology and Pharmaceutical Sciences. All investigators have access to state-of-the-art core facilities that include proteomics, genomics, bioinformatics, imaging, protein production, molecular synthesis and high throughput small molecule screening. SJCRH continues to receive support through the fundraising efforts of the American Lebanese Syrian Associated Charities (ALSAC).

The candidates will be expected to develop independent and funded research programs and to eventually become established investigators within the hospital. They will have interests in applying structural/biophysical methods to address fundamentally important biological questions. It is expected that one position will be filled by an X-ray crystallographer, and the second by an NMR spectroscopist. The new positions will be supported by generous startup funds and personnel. Candidates should have a Ph.D. and/or MD degree, at least three years of relevant postgraduate experience, and a demonstrated track record of productivity. **Applicants should send a curriculum vitae, a 1-2 page summary of research interests and future plans, and the names of three references to: Dr. Stephen W. White, Chair, Department of Structural Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105.**

www.stjude.org

St. Jude is an Equal Opportunity Employer and a Drug Free Workplace.
Candidates receiving offers of employment will be subject to preemployment drug testing and background checks.

NW12113BR

CHAIR
DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS
UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The University of Pittsburgh School of Medicine is seeking a chair for the Department of Microbiology and Molecular Genetics. The department comprises 32 primary faculty members with a focus on basic research in Microbiology, Molecular Biology, Virology, Biochemistry and Developmental Biology. In particular, candidates working within any area of Microbiology and Molecular Genetics are encouraged to apply for the chair. The successful candidate will have an outstanding record of scholarship commensurate with appointment at the rank of Full Professor with tenure, and as the William S. McElroy Professor.

The University of Pittsburgh School of Medicine is enjoying unparalleled growth in its research, clinical, and academic missions. Of more than 3,000 institutions nationwide, the University of Pittsburgh is currently ranked 7th among educational and research institutions in NIH funding. The chair of Microbiology and Molecular Genetics will have an outstanding opportunity to add further to the growth of the basic biomedical sciences in the School of Medicine.

The University of Pittsburgh is an affirmative action, equal opportunity employer. Women and members of minority groups under-represented in academia are especially encouraged to apply.

Please send a full curriculum vitae and bibliography to the MGG Chair Search Committee at:
mmg@medschool.pitt.edu NW121392R

Assistant or Associate Professor of Molecular, Cellular and Integrated Pharmacology/ Toxicology
University of California, Davis

Salary dependent on qualifications and experience. PhD or equivalent with advanced training in pharmacology/toxicology or related field; teaching aptitude/experience/potential; potential to develop outstanding independent research program that applies modern molecular, cellular, and in vivo technologies toward understanding gene-environment interactions that influence susceptibility to complex disorders of humans and animals; ability/potential to acquire extramural funding, excellent interpersonal and communication skills; ability to work with others in a collegial, team atmosphere. Desired (not required): DVM, MD or equivalent with strong research focus. To receive fullest consideration, apply by February 25, 2008, open until filled. Submit 1) letter of intent outlining special interest in the position, overall related qualifications and experience and career goals; 2) curriculum vitae, 3) 3 reprints, 4) names and addresses of three professional references to:

Isaac Pessah, Dept Chair
vm-mb@vetmed.ucdavis.edu
Dept of Molecular Biosciences
School of Veterinary Medicine
University of California, Davis
1 Shields Avenue
Davis, CA 95616
http://www.vetmed.ucdavis.edu/vmb/m.biosciences.html

E-mail applications preferred

UCD is an AA/EOE NW121381R

CLINICAL PROGRAM MANAGER - CARE AND TREATMENT

The U.S. Military HIV Research Program (USMHRP), supported by the Henry M. Jackson Foundation for the Advancement of Military Medicine, is a key contributor the President's Emergency Plan for AIDS Relief (PEPFAR/Emergency Plan) in Kenya, Nigeria, Tanzania, and Uganda. This internationally recognized program is seeking a dynamic physician to manage USMHRP's clinical programs supporting the Emergency Plan. Ensure quality clinical services are supported overseas (OCONUS) by the Walter Reed Army Institute of Research (WRAIR), as well as plan, implement and monitor clinical activities supporting the prevention, care and treatment initiatives. Must have demonstrated knowledge and ability related to HIV pathogenesis and treatment (initial infection to end stage disease palliative care); HIV transmission and prevention; Medical Education program development and management experience; Program monitoring and evaluation; Budget development, management and reporting; Excellent communication skills; troubleshooting and supervisory skills.

Must have a Medical (MD) degree with post medical graduate education in Internal Medicine and fellowship in Infectious Disease or related. A minimum of 2 years of managing hospital based Infectious Disease service; field or clinical research experience is desired; 3 to 5 years of international work experience is preferred. Incumbent will be located at Rockville, Maryland offices and travel to research/field as required on at least a semiannual basis, sponsored by the USMHRP to assist program personnel and liaise with on the ground U.S. government agencies.

Please e-mail resumes to careers@hjf.org or fax to 240-314-7334 with Job No. 203001 in subject line. The Henry M. Jackson Foundation for the Advancement of Military Medicine offers a competitive salary and generous benefits package. AA/EEO

NW1217-5R

CHAIR

DEPARTMENT OF CELL BIOLOGY AND PHYSIOLOGY

UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The University Of Pittsburgh School Of Medicine is seeking a chair for the Department of Cell Biology and Physiology. The Department comprises 22 tenure/tenure-stream faculty with a research focus on cell polarity and the trafficking of proteins and lipids, on the function and dysfunction of ion channels, on reproductive biology, and on signal transduction in diabetes and metabolism. The successful candidate must demonstrate an outstanding record of scholarship commensurate with appointment at the rank of Full Professor with tenure. An outstanding startup package has already been committed for this position, and the person who holds this position will occupy the fully endowed Richard King Mellon Chair in Cell Biology and Physiology.

The new chair will lead a significant expansion of the department and will benefit from interactions with the Center for Biological Imaging and the University of Pittsburgh Cancer Institute, and the recently established Clinical and Translational Science Institute and Drug Discovery Institute, as well as with other research units within the School of Medicine. The University of Pittsburgh School of Medicine is enjoying unparalleled growth in its research, clinical, and academic missions. The University is currently ranked 7th among educational and research institutions in NIH funding and has doubled its NIH support in the last 10 years.

Please send curriculum vitae and bibliography to the head of the **CBP Chair Search Committee (Jeffrey L. Brodsky, Ph.D.)** at: **jbrodsky@pitt.edu**

The University of Pittsburgh is an affirmative action, equal opportunity employer. Women and members of minority groups under-represented in academia are especially encouraged to apply.

NW12004BR

STONY BROOK UNIVERSITY MEDICAL CENTER

Stony Brook University Medical Center seeks highly qualified physician scientists (Assistant/Associate/Professor) to participate in developing the new Long Island Center for Clinical and Translational Sciences (LICCATS). This center will bring together clinicians and scientists from a consortium of major clinical and research institutions throughout Long Island to catalyze the translation of innovative ideas into improved clinical practice and human health. As the lead institution for this center, Stony Brook University has an extensive research portfolio. Ongoing initiatives include the recent launching of the New York Supercomputing Center and a newly created 246-acre Research and Development Park configured to house the Center of Excellence in Wireless and Information Technology and the Center for Advanced Energy, among other initiatives. LICCATS will leverage existing strengths in computational and structural biology, drug discovery and medical device design, nanotechnology, and imaging to expand its translational medicine capacities. Selected candidates will hold faculty appointments in the appropriate School of Medicine department based on the incumbents' area of expertise. **Required:** The successful candidate must have an M.D., be board eligible in their medical specialty, qualified for New York State licensure, and engaged in clinical/translational research. **Preferred:** Preference will be given to candidates with a track record of peer-reviewed NIH or similar source of funding. Qualified candidates with demonstrated abilities may be able to assume leadership positions within the institution. To qualify for an appointment as an Associate Professor or Professor, the candidates must meet the School of Medicine's criteria for Appointment, Promotion, and Tenure and must have an established reputation and record of research or scholarly activity. Competitive packages commensurate with qualifications and level of appointment will be offered with salary, protected time for research, laboratory space, and funds to initiate research programs. Review of applications will begin in January 2008 and will continue until positions are filled. Electronic submissions are preferred.

To apply online visit *www.stonybrook.edu/jobs* or send a cover letter, C.V., names, and contact information for four references to:

Marie Gelato, M.D., Ph.D., Professor of Medicine, Department of Medicine
T15-060 Health Sciences Center, Stony Brook University, SUNY
Stony Brook, NY 11794-8154

Equal Opportunity/Affirmative Action Employer.

NW121448R

VE RI TAS

Harvard University

Associate Dean for the Sciences Division

This search seeks an implementing partner for the Dean for the Sciences of the Faculty of Arts and Sciences of Harvard University as he builds the new sciences division, improves its administrative systems, and undertakes a series of science initiatives. The division is expected to pursue objectives within existing departments and centers as well as themes that bring together scientists from across the University. This recently created position will manage administrative activities spanning eleven science departments plus several research centers.

The Associate Dean position offers a significant opportunity to build an administrative career at the University. It reports to the Dean for the Sciences with a dotted line report to the Executive Dean of the Faculty of Arts and Sciences. The search is currently scheduled to conclude in the coming winter with a starting date as soon thereafter as possible.

Inquiries, referrals, and resumes should be sent (electronic submission to 3482@imsearch.com encouraged), in confidence, with a cover letter to: Liz Vago, Internal Box 3483, Isaacson, Miller, 334 Boylston Street, Suite 500, Boston, MA 02116.

For further information: 3482@imsearch.com and www.harvard.edu

Harvard University is an equal opportunity/affirmative action employer. Candidates from all backgrounds are encouraged to apply.

NW120932R

Centre for Molecular Medicine and Therapeutics

THE UNIVERSITY OF BRITISH COLUMBIA

The Centre for Molecular Medicine and Therapeutics at the University of British Columbia in Vancouver – "the world's most livable city" – seeks applications for the following position:

British Columbia Leadership Chair in Genetic Medicine

This prestigious appointment, supported by the Leading Edge Endowment Fund, will provide a unique opportunity for an internationally recognized individual to perform research in an outstanding environment, enhancing the province as a leader in genetic medicine. The successful applicant will conduct innovative research that will generate new knowledge relevant to genetic contributions to illness or molecular determinants and mechanisms of disease and/or development of novel therapeutic approaches and technologies to improve diagnosis and a treatment for disease.

The CMMT has a scientific mandate to ascertain cellular and protein function relevant to human disease as the key to improved diagnosis, treatment and prevention of health problems in children and adults. The CMMT includes outstanding infrastructure support such as a bioinformatics, expression profiling, DNA sequencing, genotyping, antibody production and world-class facilities for mouse genetics (transgenics, breeding and behavior testing) and the testing of experimental therapeutics in animal models of human disease. Located on the Children's and Women's Health Centre of BC site, the CMMT is one of the main programs of the Child and Family research Institute (www.cfri.ca), which provides comprehensive research, educational and core services to its various programs in an opportunity to interact with a broad spectrum of biomedical researchers.

The successful candidate will be appointed as a member of the full-time faculty as Professor. The applicant should ideally hold an M.D. and/or Ph.D. degree or equivalent, demonstrate excellence in teaching and have a record of recognized accomplishment. Salary will be commensurate with qualifications and experience and is subject to final budgetary approval.

UBC Faculty of Medicine *http://www.cmmt.ubc.ca*

Anticipated start date for this position is July 1, 2008
Closing date for all applications is January 31, 2008
Please send CV, names of four references, a brief statement of research interests, and a record of teaching effectiveness to:
Janet Ferraro, Human Resources Coordinator, jferraro@cmmt.ubc.ca CMMT, 950 West 28th Avenue, Vancouver, BC V5Z 4H4.

UBC and its affiliates hire on the basis of merit and are committed to employment equity. We encourage all qualified applicants to apply; however, Canadians and permanent residents of Canada will be given priority.

NW121264R

THE GEORGE WASHINGTON UNIVERSITY

GUS WEISS PROFESSORSHIP IN THEORETICAL BIOPHYSICS

The Department of Physics of The George Washington University invites applications for the Gus Weiss Professorship in Theoretical Biophysics, beginning in Fall, 2008.

Basic Qualifications: Applicants must hold a Ph.D. in Physics or a related field, have a nationally and internationally recognized and externally funded research program in theoretical and/or computational biophysics, and demonstrate strong capabilities in teaching.

Preferred Qualifications: Preference will be given to an individual whose research complements our current biophysics program, which focuses on three areas. (1) *protein structure and function* (2) *multiscale modeling of cellular control, including biological aspects of networks, signaling, and energy production*, and (3) *immune system modeling and viral dynamics*.

The successful candidate will provide leadership and vision for the growth of our biophysics group as well as lead the effort to strengthen cross-campus interdisciplinary activities in quantitative systems biology.

Application Procedure: Send a curriculum vitae, a research statement, and the names of four references to Prof. Mark E. Reeves (reevesme@gwu.edu), Chair, Search Committee, Department of Physics, GWU, Washington, DC 20052

Only complete applications will be considered: their review will begin on February 1, 2008 and will continue until the position is filled.

The George Washington University is an equal opportunity, affirmative-action employer.

NW 21419R

CHARITÉ

UNIVERSITÄTSMEDIZIN BERLIN

Gliedkörperschaft der Freien Universität und der Humboldt-Universität zu Berlin

The Medical Faculty of the CHARITÉ - UNIVERSITÄTSMEDIZIN BERLIN Charité Centrum 2 (Institute for Biochemistry, CCM and Institute for Biochemistry and Molecular Biology, CBF) invites applications for the following positions:

2 Tenure-Track Professorships in Biochemistry (BesGr. W2)

(Code number: 10.07)

- ***Area of responsibility:*** The field of biochemistry in research and teaching
- ***Qualifications:*** A postdoctoral thesis (Habilitation) or the equivalent are required according to § 100 of the Berlin Higher Education Act (BerlHG)
- ***Requirements:*** We are looking for enthusiastic and highly motivated individuals with outstanding track records in any area of biochemistry, cell biology and/or molecular biology. Experience in the acquisition, administration and application of extramurally funded projects is expected. The candidates' research interests should be compatible with the Charité scientific program (www.charite.de). They must be willing to closely cooperate with the scientific departments of the Humboldt Universität zu Berlin and the Freie Universität Berlin as well as external university institutions and research associations.

The candidates should have excellent didactic skills and a high dedication to teaching. Experience in imparting knowledge in biochemistry to undergraduate medical students is desirable.

The positions are tenure track; they are initially limited to 5 years with options for extension or tenure depending on positive evaluation.

Applications of women are specifically invited. In the case of equivalent qualification, competence, and specific achievements, women will be considered on preferential terms within the framework of legal possibilities.

Handicapped candidates with equivalent qualifications will be given preference.

Applications should be sent within **4 weeks** after publication of this advertisement including a CV, list of grants and publications, a summary of current and planned research objectives and other relevant material (see: http://www.charite.de/jobs/prof.htm) to: **Charité - Universitätsmedizin Berlin, Der Dekan, Prof. Dr. Martin Paul, Charitéplatz 1, 10117 Berlin, Germany.**

UAB

SCHOOL OF MEDICINE

Department of Medicine
Division of Cardiovascular Disease

INSTRUCTOR OF MEDICINE

The University of Alabama at Birmingham, Division of Cardiovascular Disease is seeking applicants with a Ph.D. or M.D. for the position of Instructor. This is a non-tenure earning faculty position. Candidates must be capable of independently designing and conducting experiments and preparing and submitting data for publication. It is expected that the successful candidate will be able to secure independent extramural funding to support ongoing research. Individuals interested in this position should have experience in small animal surgery and be able to work in both *in vitro* and *in vivo*.

Interested candidates should send CV, three letters of reference, and a brief statement of your interest to:
Robert C. Bourge, M.D., Director
1900 University Blvd.
UAB Station, Birmingham
AL 35294

UAB is an equal opportunity/ affirmative action employer

NW120581R

Novo Nordisk

Novo Nordisk
Haemostasis Biology
Research Scientist

We are focused on understanding the biology of haemostasis, coagulation and fibrinolysis, with special emphasis on functional aspects of haemostatic proteins. The goal is to develop novel protein therapeutics for treatment of haemophilia. You hold a Ph.D/equivalent degree and have solid experience with biological assays. Ref: NN37572 Research Scientist.

Contact

Brit Bitow Sorensen
Tel: +45 4443 4353/+45 3075 4353
www.novonordisk.com

W121611RL

Forschungszentrum Karlsruhe in der Helmholtz-Gemeinschaft

Forschungszentrum Karlsruhe
Special field: Nanotechnology
Scientist (m/f)

Main tasks within the framework of the EU Project "NANOHy" are synthesis, functionalisation and characterization of nanocarbons and nanocomposites by using physisorption, X-ray diffraction, vibrational spectroscopy techniques, and thermal analysis; work is done under inert conditions; three-year fixed-term contract.

Contact

name Dr. Fichtner
Tel: 00 49 7247 82-5340
www.fzk.de Email: fichtner@mt.fzk.de

W121497RL

University of Oxford

DEPARTMENT OF EARTH SCIENCES

Research Associate/Fellow in Mass Spectrometry

The Department seeks a Research Associate or Fellow in Mass Spectrometry to conduct and assist research in the Department of Earth Sciences of Oxford University.

The geochemistry research programs include various aspects of earth, environmental and planetary sciences and are supported by a wide range of analytical instrumentation that provides Oxford with one of the best-equipped facilities in the world. With new laboratories recently constructed and the acquisition of further mass spectrometers, we seek talented and enthusiastic individuals to maintain and develop a range of mass spectrometers and who are keen to work in a dynamic, interactive and technically innovative geochemistry research facility.

Dependant on skills and qualifications, the successful candidate will be appointed either as a Research Associate (£26,666 - £32,796 p.a.), or a Research Fellow (£33,779 - £40,336 p.a.). The appointment will be for three years in the first instance.

Letters of application with a full CV and the name and address of three referees (at least one of whom should be a current or previous employer) should be sent to Caroline Hutchings, Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR or E-mailed to: caroline.hutchings@earth.ox.ac.uk and received no later than Thursday 17 January 2008, quoting reference number DG/07/015. Further particulars are available from www.earth.ox.ac.uk/department/massspec.pdf

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U121187P

www.ox.ac.uk/jobs

HEINRICH HEINE UNIVERSITÄT DÜSSELDORF

In der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität ist eine

W3-Professur für Mikrobiologie

(Nachfolge Prof. Dr. C. Hollenberg)

zum 01.10.2008 zu besetzen

➢ Die Heinrich-Heine-Universität bildet mit über 16.000 Studentinnen und Studenten den Schwerpunkt des Hochschul- und Wissenschaftsstandortes Düsseldorf.

➢ Mit ihren fünf Fakultäten, der Juristischen Fakultät, der Medizinischen Fakultät, der Philosophischen Fakultät, der Mathematisch-Naturwissenschaftlichen Fakultät und der Wirtschaftswissenschaftlichen Fakultät, fördert sie die enge interdisziplinäre Zusammenarbeit auf regionaler und internationaler Ebene.

➢ Die Bewerberinnen/Bewerber sollen in aktuellen Gebieten der eukaryotischen Mikrobiologie forschen. Eine Mitarbeit in den relevanten Forschergruppen und Sonderforschungsbereichen der Heinrich-Heine-Universität Düsseldorf und/oder im Zentrum für Mikrobielle Biotechnologie am Forschungszentrum Jülich wird erwartet. In der Lehre soll das Fach Mikrobiologie in den Studiengängen von Biologie, Biochemie und Medizin anteilig vertreten werden.

➢ Einstellungsvoraussetzungen sind neben den allgemeinen dienstrechtlichen Voraussetzungen gem. § 36 des Gesetzes über die Hochschulen des Landes Nordrhein-Westfalen insbesondere pädagogische Eignung, besondere Befähigung zu wissenschaftlicher Arbeit sowie zusätzliche wissenschaftliche Leistungen.

➢ Die Universität strebt an, den Anteil der Frauen am wissenschaftlichen Personal zu erhöhen, und begrüßt daher besonders Bewerbungen von Wissenschaftlerinnen.

Frauen werden bei gleicher Eignung, Befähigung und fachlicher Leistung bevorzugt berücksichtigt, sofern nicht in der Person eines Mitbewerbers liegende Gründe überwiegen.

Die Bewerbung geeigneter Schwerbehinderter ist erwünscht.

➢ Bewerbungen mit den üblichen Unterlagen, einer Übersicht der Drittmittel der letzten 5 Jahre, den Sonderdrucken von 5 ausgewählten Publikationen sowie einer kurzen Darstellung der bisherigen Lehrtätigkeit und der Forschungsperspektive werden bis zum 15.02.2008 an die unten stehende Adresse erbeten. Wir bitten die kompletten Bewerbungsunterlagen in Papierform wie auch als PDF Datei einzureichen.

TOTAL E-QUALITY

Dekan der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1 · D-40225 Düsseldorf

W121353R

ALBERT-LUDWIGS-UNIVERSITÄT FREIBURG

At the Faculty of Medicine of the Albert-Ludwigs-University of Freiburg i. Br. a

W3 professor position (successor of Prof. Dr. Fleckenstein-Grün)

is available immediately. The position is located within the Department I of the Institute of Physiology (Head of Department: Prof. Dr. Peter Jonas). The person to be recruited is expected to have a research focus in Cellular Neurophysiology and to participate in the new Collaborative Research Center 780 "Synaptic Mechanisms of Neuronal Network Function".

Applicants should have a final exam in Natural Sciences or Medicine, MD or PhD degree, and habilitation or equivalent scientific qualification. The new professor is expected to contribute to teaching in Physiology and to organize the practical course Physiology for medical students.

Internationally recognized research achievements, funding, experience in leading a research group, and experience in supervision of MD and PhD students are expected. Teaching expertise in all areas of Physiology is required.

The W 3-position is tenured. However, in accordance with the State University Law (LHG), if this is the candidate's first appointment to a professorship, employment will be initially limited to three years with prolongation subject to evaluation. Exceptions are possible, particularly in the case of applications from abroad or from a non-academic institution.

The University of Freiburg is an equal opportunity employer. Applications of women are strongly encouraged. Handicapped candidates with equivalent qualifications will be given preference as well.

To obtain application forms, please send an E-Mail to dekanat-professuren@uniklinik-freiburg.de. Completed applications together with all pertinent documents should be sent no later than February 15th,2008 to the Dean of the Faculty of Medicine, Prof. Dr. med. Christoph Peters, Albert-Ludwigs-University, D-79085 Freiburg, Germany (Phone: ++49-761-270-7235/7234, Fax: ++49-761-270-7236).

W121522R

---

www.cam.ac.uk/jobs/
A world of opportunities

UNIVERSITY OF CAMBRIDGE

Research Associate

Department of Oncology
£25,134 - £32,796 pa
Limit of tenure: Three years

You will work in the Cell Cycle and Development Group housed within the Hutchison/MRC Research Centre. The laboratory aims to study the regulation of cell cycle and differentiation during early embryogenesis in both Xenopus embryos and mammalian cells, as a basis for understanding how these processes are regulated in normal development and how they are perturbed in cancer.

You will be given the opportunity to work on a project investigating ubiquitin-mediated proteolysis of a key transcription factor that drives differentiation of the endocrine pancreas. The project will involve biochemical, cell biological and embryological techniques. You must have a PhD in molecular cell biology or related subject, and experience of biochemistry, cell culture and/or embryology would be a significant advantage. Good written and oral communication skills are essential, and a proven track record of published research is highly desirable.

Requests for further information, and informal enquiries, should be addressed to Dr Anna Philpott, e-mail: ap113@cam.ac.uk

Completed applications consisting of a PD18 form (please complete parts I, IIa, III), CV and the names and addresses of two referees should be sent to Judith Nial via email: jln23@cam.ac.uk or to Department of Oncology, Hutchison/MRC Research Centre, Box 197, Hills Road, Cambridge, CB2 0XZ.

Please quote reference: RD02784. Closing date: 1 February 2008.

The University is committed to Equality of Opportunity.

J121402R

Universitätsklinikum Hamburg-Eppendorf

The Faculty of Medicine of the University of Hamburg - Centre for Molecular Neurobiology (ZMNH) - invites applications for the position of a

Full Professor (W3) for "Molecular and Cellular Neurobiology"

Vacancy NO. FB04-78/3

The appointed scientist will be Director of the Institute for Developmental Neurobiology and will be involved in research and teaching.

Possible areas of research preferably include neurodegeneration, neuroregeneration, molecular mechanism in cognition, metabolism in the nervous system.

Teaching (4 hours per week) will be within an existing molecular biology graduate program.

The ZMNH comprises five institutes (Developmental Neurobiology, Neuropathobiology, Neuronal Signal Transduction, Biosynthesis of Neuronal Structures, Neuroimmunology and Clinical Multiple Sclerosis Research), currently four independent junior research groups, and six central service units (mass spectrometry, morphology, DNA sequencing, transgenic mouse facility, library, and computer group). For more information see http://www.zmnh.uni-hamburg.de

Conditions of employment: The position is open to German and foreign nationalities. Experience in leading a research group and in raising grant money is required.

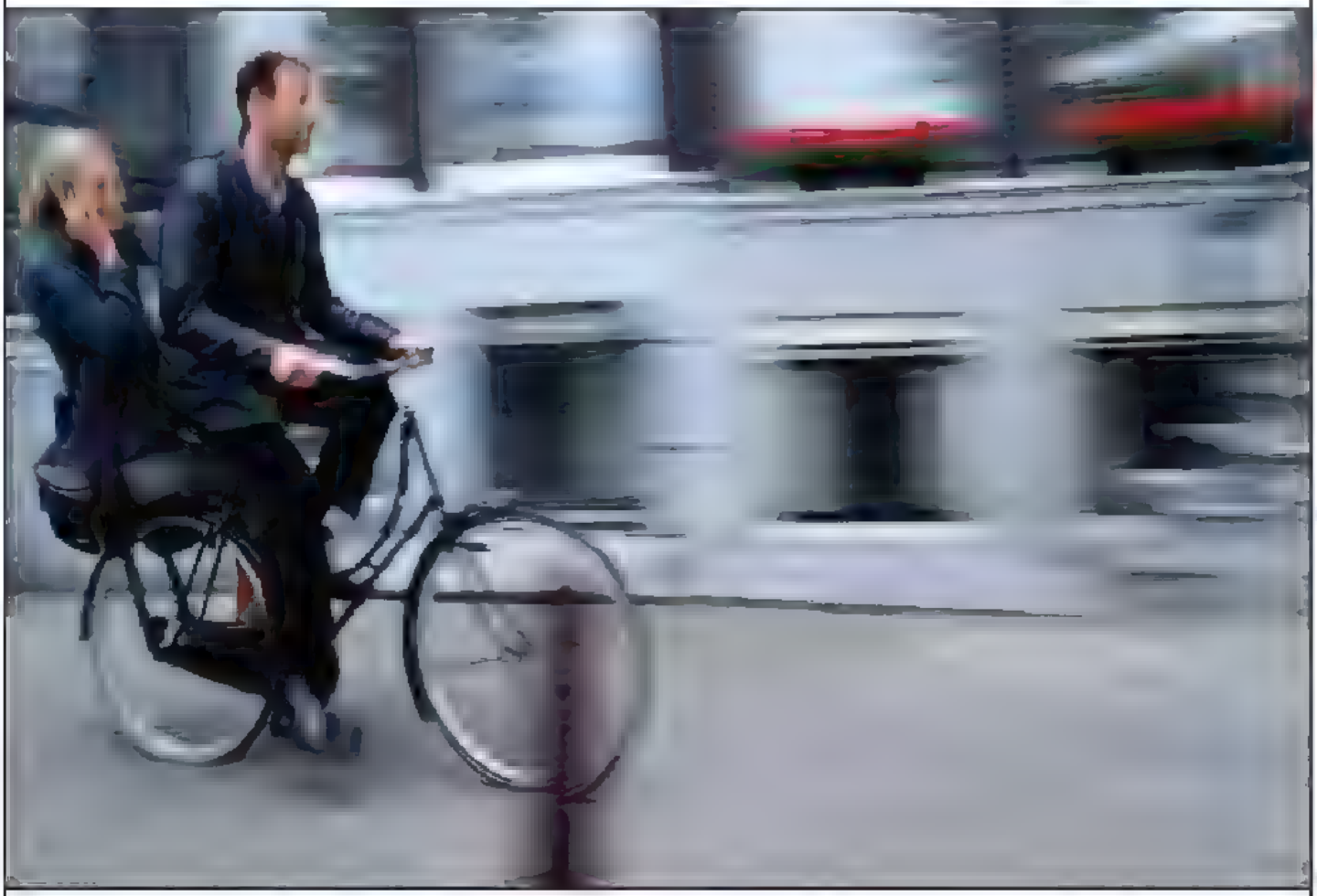
The University of Hamburg is committed to employment equity and women are particularly encouraged to apply. Applicants with disabilities will be preferentially considered within the current legal regulations.

Employment will be according to the regulations of the University of Hamburg (§15). Applications with cv, list of publications, current research funding, teaching experience and a short outline of planned research projects should be submitted in triplicate by **February,14th 2008** quoting the number **FB04-78/3** to the Dean of the Faculty of Medicine of the University of Hamburg, Fakultätsservice -SV-, Martinistraße 52, 20246 Hamburg/GERMANY

W121352R

Bringing jobseekers and recruiters together.

Academic • Industry • Government



events • announcements • prospects • regions • careers & recruitment
spotlight • special reports • job channels • focus • movers

Search now at **naturejobs.com**

naturejobs
making science work

nature publishing group npg

BIPM

Bureau International des Poids & Mesures

Head of Publications

Location

The BIPM (International Bureau of Weights and Measures) in Sèvres, France, is an intergovernmental organization whose mandate is to provide the basis for a coherent system of measurements throughout the world, traceable to the International System of Units (SI). It has an international staff of over 70 and an annual budget of about 10 million euros. Further information about the BIPM and *Metrologia* can be found on the website www.bipm.org

The closing date is 25 January 2008

Duties

The BIPM has two main groups of publications: one is a continuing series of reports of meetings on metrology held at the BIPM; the other is *Metrologia*, an international journal with an Impact Factor of 1.657 dealing with pure and applied metrology. Since 1 January 2003, *Metrologia* has been published in partnership with the Institute of Physics Publishing (UK).

The Head of Publications is Editor of *Metrologia*, and in addition has an important role in the internal review of BIPM staff publications prior to their submission to external journals in the open scientific literature.

The appointee is moreover expected to take an active interest in and to offer advice on, and take part in, the promotion of the BIPM's scientific programme using the web and other suitable media.

The Head of Publications is supervising the Publications section, which comprises three additional staff: a full time webmaster with responsibility for the BIPM website, and the BIPM's IT group, currently staffed by two specialists.

Qualifications

Applicants should have:

- a PhD and a broad knowledge of the physical sciences;
- an extensive experience as a research physicist or chemist as well as some direct knowledge of the editorial aspects of journal publication;
- an excellent knowledge of English and a working knowledge of French, since many BIPM publications are bilingual;
- management experience;
- an awareness of IT networks and systems;
- the ability to work in a multicultural environment and to maintain good working relations inside and outside the organization.

Employment conditions

The BIPM offers salaries and terms of employment in accordance to its Staff Rules, which is comparable to those of other international organizations based in France and operates its own contributory pension scheme.

Applications

The BIPM encourages applications from both women and men with relevant qualifications. A full Curriculum Vitae (C.V.) and covering letter - including the names of two referees who will be asked to comment upon the candidate's suitability for the post - should be sent by paper mail to the Director, BIPM, Pavillon de Breteuil, F-92312 Sèvres Cedex, France, by 25 January 2008.

The successful applicant would be expected to take up the position before the end of April 2008.

W121462R

Head of Online User Experience

Nature.com is one of the foremost scientific destinations on the web. As well delivering the unsurpassed scientific content for which *Nature* is renowned it also provides a range of innovative and award-winning services including databases, blogs, discussion forums, document-sharing services and podcasts. This explosion of online creativity reflects NPG's strong believe that the new online world can be harnessed to support scientific communication in a wide variety of ways that have not been possible before, and that many of these represent new business opportunities. We are now seeking a technologically and scientifically astute individual to coordinate these activities for the benefit of scientists, and hence to create the definitive online scientific service. This position will be based in either our London or New York office.

This role will focus on maximising the usefulness of *Nature.com* and NPG's other websites. As such it will involve acting as the scientists' representative within the company for issues of online usability, and working across internal departments to implement new or improved online features. The person responsible will need to understand in detail how professional scientists use information, and how *Nature.com* can serve these needs more effectively, with the ultimate aim of increasing usage and user satisfaction.

We are therefore seeking a candidate with a detailed knowledge of best practice on the web, a general familiarity with the benefits and limitations of key web technologies, the ability to work effectively with a wide variety of colleagues at every level, and willingness, flexibility and energy in taking initiative and assuming responsibility. A demonstrable interest in science is essential and an educational background in science would be preferable.

To apply, please send your CV and covering letter (quoting reference NPG/LON/772), stating your current salary to Kerrie Willsher, Personnel, Macmillan Publishers at londonpersonnel@ macmillan.co.uk

Closing Date. 31st January 2008

nature publishing group

IN120808R

Corrosive, uncompromising opinions and views on workplace issues affecting YOU.

naturejobs

MRC Medical Research Council

MRC Integrative - Toxicology Training Partnership (ITTP)

The Medical Research Council is launching a new initiative aimed at improving and securing capacity in toxicology. The emphasis of the ITTP will be on aligning modern cellular and molecular biology with other fundamental and health-related disciplines relevant to Toxicology to provide an integrated approach to research and training.

Funding for up to 6 PhD/DPhil Studentships and 2 Career Development Fellowships is available for 2008. The studentships will be of 4 years duration and will include taught courses in a wide range of aspects of toxicology as well as pertinent advances in molecular sciences. The programme will be managed by the MRC Toxicology Unit, University of Leicester, UK.

Applications are sought from academic departments, MRC Institutes and Units, and charity-funded institutes. Collaborative partnership between researchers across disciplines and universities and other relevant organisations, including industry and Government agencies, are particularly encouraged.

Application forms and further details are available at http://www.le.ac.uk/mrctox/MRCTox/ittp.htm. Further information can be obtained by contacting Dr Andy Smith at ags5@le.ac.uk

Closing dates for receipt of completed applications for Studentships are 31st January 2008 and Fellowships 31st March 2008.

U121734R

deafness research uk

UCL

UCL The Ear Institute

Senior Research Associate

"Opportunity for emerging PIs in hearing research"

The UCL Ear Institute is a multi-disciplinary facility which opened in January 2005 with the remit of "understanding hearing and fighting deafness" Following the successful appointment of two Deafness Research-UK Research Fellows, the Institute is looking to recruit a third new investigator for a 3-year Senior Research Associate position, available from September 1st 2008 The successful applicant will be given support and encouragement to obtain external funding during the term of the appointment with the aim of becoming a principal investigator at the Ear nstitute

The focus of this particular search is for outstanding individuals whose expertise lies in one or more of the following fields of endeavour; electrophysiology including cochlear physiology, cochlear biophysics and modelling, psychoacoustics, and neuroscience of the central auditory system. Outstanding candidates whose research nterests lie in other areas of interest, including human genetics, molecular and cellular biology, developmental biology, cell physiology, audiological science or medical imaging and its clinical interface are also encouraged to apply

Salary will be on Grade 8 of the University College London Salary Scale which ranges from £34,793 - £41,545 p.a. plus £2,649 London Weighting p.a

Further details and instructions as to how to apply can be found on our website: www.ucl.ac.uk/ear/jobs/jobs htm.

Informal email enquiries may be made to the Institute Director, Professor David McAlpine, on d mcalpine@ucl.ac.uk

Closing date: 31st January 2008

UCL Taking Action For Equality

U12 388R

SPINAL RESEARCH

Nathalie Rose Barr PhD Studentships

Call for Proposals

The International Spinal Research Trust is a UK based medical research charity (no. 281325) with the sole purpose of funding research aimed at resolving the non- or partial functioning of the injured spinal cord

We invite senior scientists (supervisors) at UK institutions to submit proposals for the support of PhD studentship projects, up to four years for science graduates or three years for those with clinical qualifications

Application forms are available on request, further information on our research strategy and other requirements can be found on our website

Closing date for receipt of applications is **Monday 11th February 2008.**

www.spinal-research.org E-mail: **research@spinal-research.org**

U121276A

28th Blankenese Conference

Minerva-Gentner Symposium

Sensory Signaling and Information Processing

May 25-29, 2008

Hamburg-Blankenese, Germany

Organising Committee: Doron Lancet, Wolfgang Meyerhof and Dietmar Richter

Topics: Taste olfaction mechanoperception vision audition information processing in sensory pathways

Keynote Speaker

L. Buck, Seattle

Speakers

E. Ahissar, Rehovot	H. Hatt, Bochum	P. Mombaerts, Frankfurt
K. Avraham, Tel Aviv	S. Herness, Columbus	M. Naim, Rehovot
E. Barkai Haifa	J. Hildebrand, Tucson	K. Palczewski, Cleveland
H. Breer, Stuttgart	B. Kaupp. Jülich	Z. Selinger Jerusalem
D. Drayna, Rockville	S. Korsching, Cologne	I. Segev, Jerusalem
Y. Dudai, Rehovot	D. Lancet, Rehovot	G. Shepherd, New Haven
T. Finger, Aurora	G. Lewin, Berlin	W. Singer, Frankfurt
S. Firestein, New York	R. Malach, Rehovot	N. Sobel, Rehovot
R. Friedrich, Basel	R. Margolskee, New York	B. Vaadia, Jerusalem
G. Galizia, Konstanz	W. Meyerhof, Potsdam	Z. Wollberg, Tel Aviv
Y. Gilad, Chicago	B. Minke Jerusalem	F. Zufall, Homburg

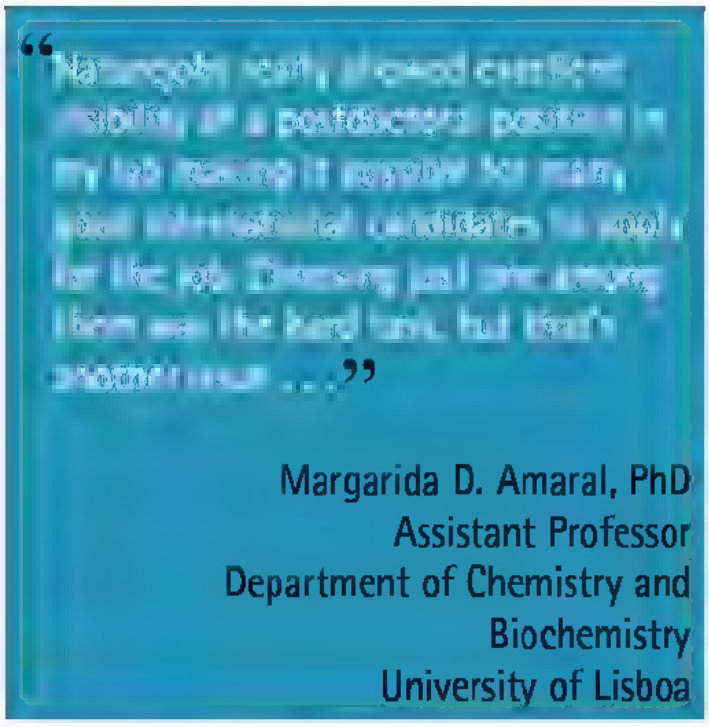
Call for Abstracts

You are invited to submit one page abstracts for poster presentations. A number of abstracts will be selected for oral presentations.

For registration see http://www.zmnh.uni-hamburg.de/blankenese conferences/

Deadline for submissions: March 15th 2008

W11886E



REPROGRAPHICS: J Jays Limited, Essex SS2 5SF UK and **The Charlesworth Group,** Wakefield, UK
PRINTED BY: St. Ives Plymouth Ltd. UK, **Publishers Press,** Lebanon Junction, Ky, USA and **Obun Printing Co. Inc,** Tokyo, Japan

Nature Publishing Group and The New York Academy of Sciences present

Nature Chemical Biology Symposium: Chemical Neurobiology

The second *Nature Chemical Biology* Symposium: Chemical Neurobiology will explore how chemists and biologists are using the tools and philosophy of chemical biology to understand the molecular basis of neuronal function. The two-day meeting will comprise a series of four scientific sessions that look at distinct molecular functions of a neuron and concludes with a keynote session featuring Linda Buck, a pioneer in the field of neuroscience.

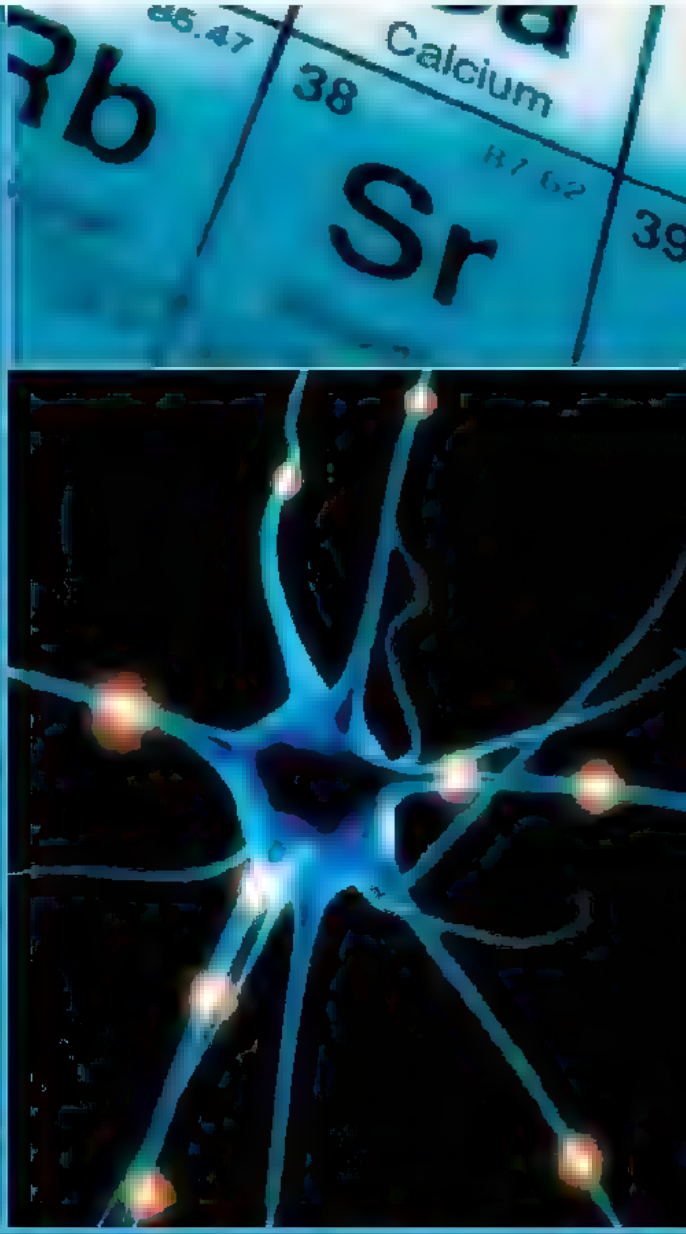
February 22-23, 2008
The New York Academy of Sciences
New York, USA

[redacted]

KEYNOTE SPEAKER
[redacted]

SPEAKERS
[redacted]

[redacted]
http://www.nature.com/natureconferences/nchembio2008



nature conferences

Organizers

nature chemical biology

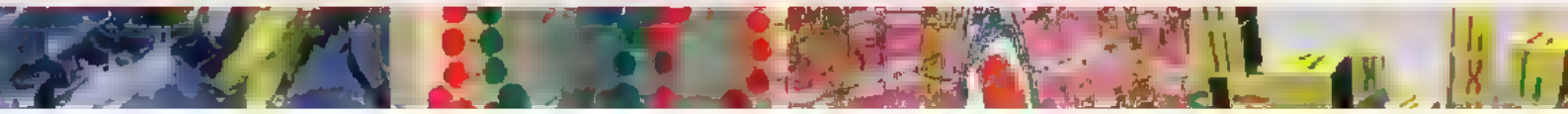
The New York Academy of Sciences

nature publishing group npg

IN11082BE

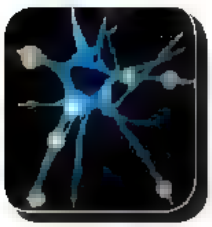
nature conferences

Unique events committed to scientific excellence



Come and join us in 2008…

Miami Winter Symposium
Regulatory RNA in Biology and Human Health
February 2-6, 2008
Miami Beach, FL, USA
www.nature.com/natureconferences/miami/MWS2008



***Nature Chemical Biology* Symposium 2008**
Chemical Neurobiology
February 22-23, 2008
New York, NY, USA
www.nature.com/natureconferences/nchembio2008

Emergence & Convergence mini-symposium
Epigenetics and Behavior
March 31, 2008
Houston, TX, USA
www.nature.com/natureconferences/eandc/epigen

XX International Congress of Genetics
Genetics – Understanding Living Things
July 12-17
Berlin, Germany
www.nature.com/natureconferences/icg2008

And more…

Check the *natureconferences* website often for new conferences, conference updates, speaker lists, registration and application details, and more information.
www.nature.com/natureconferences

nature publishing group npg

Max Planck Society and *Nature Immunology* present:

Determinism and plasticity of T lymphocytes

A Ringberg Colloquium

Increasing evidence suggests that reciprocal signaling pathways regulate the development of various T subsets, in particular T helper and T regulatory cells. Consequences of how these regulatory pathways operate can either exacerbate or ameliorate disease and infection; of paramount importance to outcome are intrinsic (gene programs) and extrinsic factors (cytokines) that determine which subset of T cell develops. Discussions will approach these issues from different angles, linking a broad range of experimental approaches and cellular and molecular mechanisms with chronic inflammation and disease.

This is a closed meeting with a non-traditional, highly interactive, discussion-driven format, in which attendees are chosen by invitation or application. The aim is to stimulate conceptual breakthroughs leading to advancement in the fields of chronic inflammation and control of infection mediated by T lymphocytes.

February 10-13, 2008
Schloss Ringberg
Tegernsee, Germany

ORGANIZERS
Stefan H.E. Kaufmann (Max Planck Institute for Infection Biology, Germany)
Douglas Braaten (*Nature Immunology*, USA)
Vijay K. Kuchroo (Brigham and Women's Hospital; Harvard University, USA)
Jamie Wilson (*Nature Immunology*, USA)
Harmut Wekerle (Max Planck Institute of Neurobiology, Germany)
Rudolf Grosschedl (Max Planck Institute of Immunobiology, Germany)

SPEAKERS
Abul Abbas (University of California - San Francisco, USA)
Alan Aderem (Institute for Systems Biology, USA)
Rafi Ahmed (Emory University, USA)
Christophe Benoist (Joslin Diabetes Center, USA)
Michael J. Bevan (University of Washington - Seattle, USA)
Jeffery A. Bluestone (University of California - San Francisco, USA)
Dan Cua (Schering-Plough Biopharma, USA)
Chen Dong (MD Anderson Cancer Center, USA)
Ronald Germain (National Institutes of Health, USA)
Christopher Hunter (University of Pennsylvania, USA)
Robert Kastelein (Schering-Plough Biopharma, USA)
Dan R. Littman (New York University School of Medicine, USA)
Pierre Miossec (Hôpital Edouard Herriot, France)
Ken M. Murphy (Washington University School of Medicine, USA)
John O'Shea (National Institutes of Health, USA)
Fiona Powrie (University of Oxford, UK)
Anjana Rao (Harvard Medical School, USA)
Steven L. Reiner (University of Pennsylvania, USA)
Alexander Rudensky (University of Washington - Seattle, USA)
Brigitta Stockinger (National Institute for Medical Research, UK)
Casey Weaver (University of Alabama - Birmingham, USA)
Steven F. Ziegler (Benaroya Research Institute, USA)

In addition to the invited speakers, 10 participants will be chosen by application

To apply and for more information please visit:
www.nature.com/natureconferences/ringberg2008

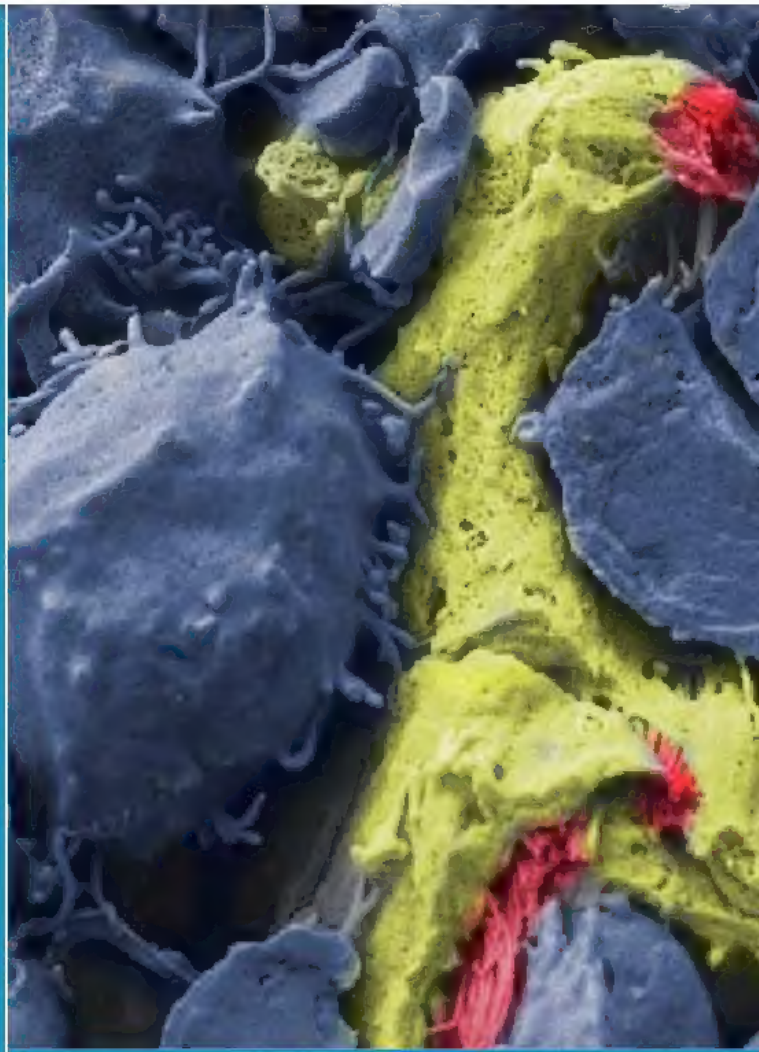


Image is kindly provided by Jean Pierre Laugier (University of Nice, France), Marc Bajénoff (National Institutes of Health, USA) and Ronald N. Germain (National Institutes of Health, USA)

a nature conference

Organizers

nature immunology

MAX-PLANCK-GESELLSCHAFT

Sponsored by

Schering-Plough

The Schering-Plough® name and S-P Logo® are registered trademarks of Schering Corporation, used with permission.

IN113583E

When Britney Spears comes to my lab

Now that's what I call pop culture!

Vince LiCata

FOR IMMEDIATE RELEASE: Louisiana native Britney Spears has been considering attending a few classes at the Louisiana State University (LSU) in Baton Rouge, not far from her hometown of Kentwood.

When Britney Spears comes to LSU she'll be wearing a silver strapless stretch top that doesn't show too much of her belly (unless she actually moves her arms), and black Capri pants with a little dip in the waistband. Soon after she arrives she'll start working as a student researcher in my laboratory, because, to tell the truth, Intro Biology will be more interesting than she expected. Cutting up dead animals in the student lab will give her an odd, heady rush that she'll find slightly disorientating and mildly pleasant. She'll feel driven to learn more about real scientific research.

I'll teach Britney not to chew gum over the experiments, how to untangle her hair from the pH meter, and what the label "use only with adequate ventilation" means. Soon she'll get her very own research project, most likely one of our diabetes projects, as the match between a pop star and research on abnormal sugar and lipid metabolism should work well. Britney will pump out a lot of good data (she is something of a workaholic), but gradually, with her music, her intermittent marriages and pregnancies, not to mention her classes, the amount of time she spends in lab will begin to dwindle. I'll give her my standard lecture about commitment, but in the end, Britney will decide to put in more hours at the lab for the same reason all good scientists do: because she likes it.

As Britney becomes more adept and independent in the lab, injuries will become less frequent, as will damage to the equipment. A particularly harrowing incident involving an autotitrator and a Lycra stretch top will induce her to dress more conservatively. Her data will be solid, reproducible and publication-quality. She'll use vacations and breaks to record new songs, and tour during summers. Otherwise, she'll spend a lot of time in the lab, even when it sometimes means flying in and out of town the same day.

After the initial shock, new students will quickly learn that Britney is one of the most productive members of our group. All lab members will learn how to expel annoying reporters, politely and efficiently. The assortment of groupies who will constantly hang around outside our laboratory door will be, for the most part, well-behaved.

Britney's contributions to science will reach far beyond my laboratory. Britney will engender an unprecedented level of public interest in basic science. Funding in all areas of research will reach miraculous new levels.

JACEY

Britney will teach the public that you have to understand the fundamentals of a system before you can even think about curing a disease. This won't happen overnight. In fact, the first time she's asked about her research on *Entertainment Tonight* she'll mispronounce the word 'adipocyte'. But soon her public discourse will become error-free and on-point. The number of young people and other celebrities entering science careers will skyrocket.

After LSU, Britney will go on to earn her PhD from Harvard. The same month Britney defends her dissertation, she will co-host the Grammys wearing a semi-transparent version of a graduation gown and PhD hood. During her postdoctoral work at the Pasteur Institute, she'll adapt well to the French diurnal rhythms: sauntering into the lab around 11, and working till dinner at 10 in her favourite Montparnasse cafe. Her work with whole-animal responses to the antidiabetic treatments she will have developed in graduate school will attract international attention. French journalists, like the Americans before them, will come to accept the fact that they will have to learn some basic biochemistry if they want an interview with Dr Spears.

Britney will spend the next few years setting up her own laboratory and in-lab recording studio at her first faculty position in the Biochemistry Department at St Jude's Children's Hospital in Memphis, Tennessee. Will her colleagues resent her skimpy outfits, that garish eyeliner, and the constantly blaring pop music coming from her lab? Some will, but most will realize that beneath that spandex and body glitter is the heart of a dedicated scientist. Britney's work will uncover promising new avenues for treating diabetes. Unlike many scientists, she'll have to hire security when she gives a research talk. Her yearly FBI report will show that the majority of her stalkers have PhDs.

One night, not many years later, Britney will be sitting alone in a diner on Elvis Presley Boulevard, nursing a cup of coffee, and trying to stay awake in order to go help a student take a late-night time-point. She'll be looking out the window and wondering what her life would have been like if she hadn't spent so much time in the lab and had stayed more involved with her music. The window will reflect the profiles of a woman and a boy sitting in the booth in front of her. The boy will look up from his magazine.

"Mom, what's diabetes?"

"Well, honey, it's a serious disease that has to do with your body not properly digesting sugar. It can make you go blind, or have to have your legs amputated or even kill you."

"Eww. What if I got that?"

"Well, you don't have it, honey, but even if you did, I heard they have a lot of new treatments for it now. I heard Britney Spears works on it."

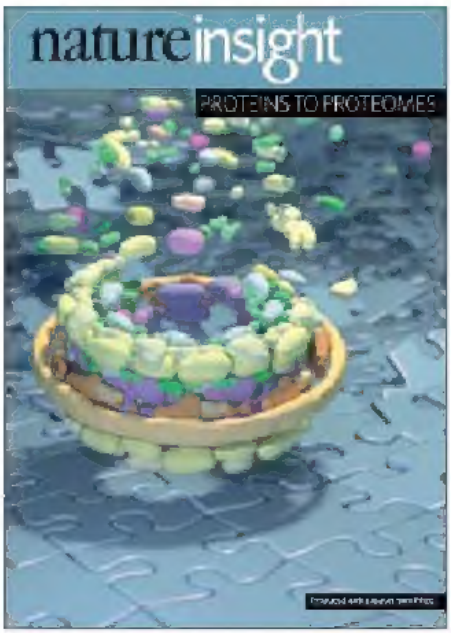
The boy will go back to his reading. Britney will take another sip of coffee. Looking up, she'll catch the eye of the waitress, and order a slice of pie. She's supposed to be on a diet, but she hasn't shown her belly in public for years, and there's still 20 minutes before she has to be back in the lab. ■

Vince LiCata is an associate professor of biological sciences at LSU, studying protein structure and function. He also writes plays, which have been produced in several US cities.

nature insight

PROTEINS TO PROTEOMES

Published in the Dec 13 issue of *Nature* and available free online for 6 months



This Insight features truly outstanding Reviews, written by an all-star panel of leaders in the field. Including the most vibrant research on how various proteins interact with each other to form the larger machines that get our cells running, including how such basic research translates into drug discovery.

Biochemists began by taking cells apart, and have worked hard to isolate cellular components from each other, so as to be able to understand elementary functions. After almost to two centuries of intense labour, and now that full part lists (genomes) have been completed, they are faced with the problem of understanding how all those parts work together as a coherent ensemble. This Insight highlights some of the most exciting new discoveries involving the protein world.

Produced with support from

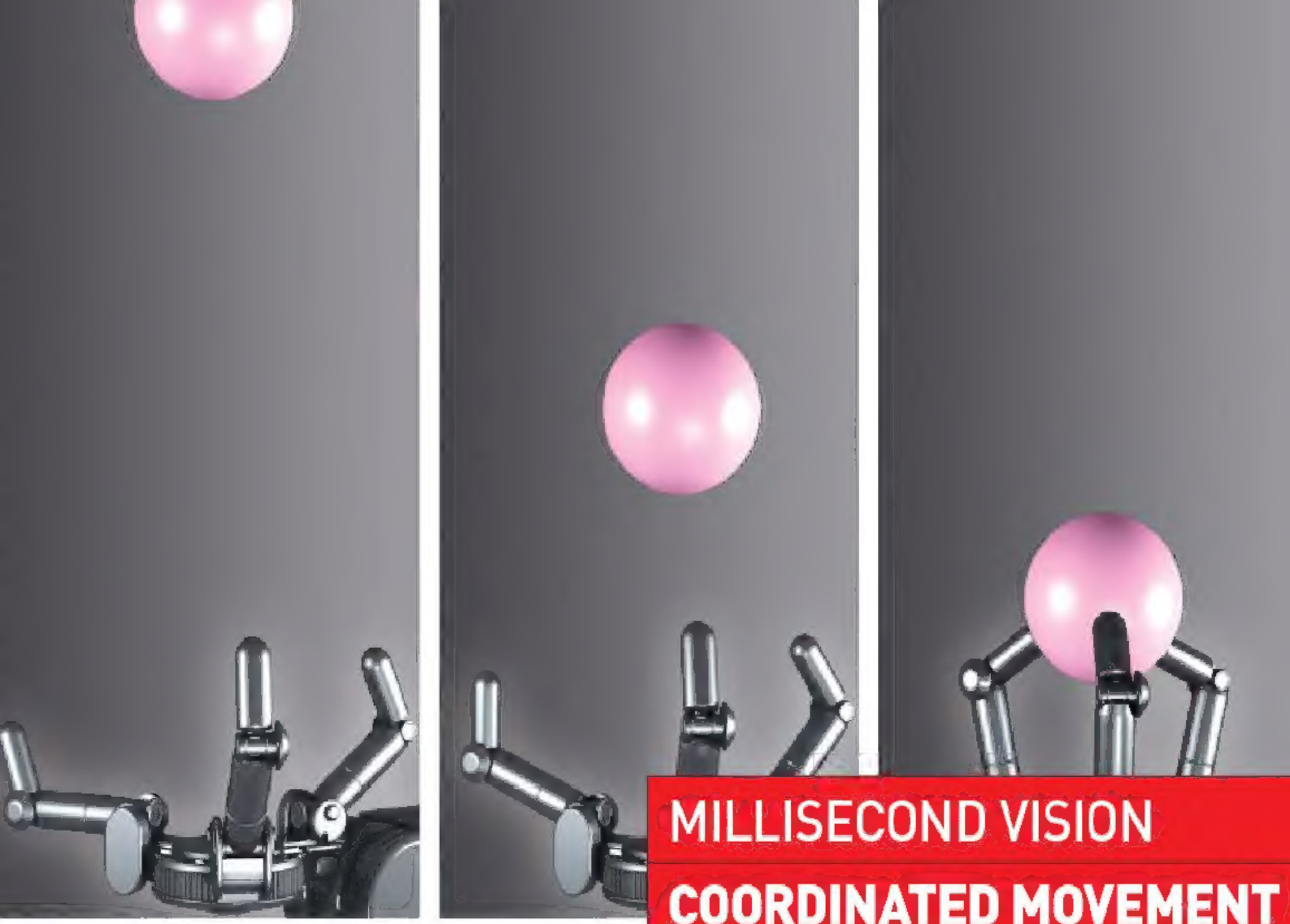
Pfizer

Read the Insight in the Dec 13 issue of *Nature* or access it online at:

www.nature.com/nature/supplements/insights

nature

nature publishing group npg



MILLISECOND VISION
COORDINATED MOVEMENT

Hamamatsu Photonics Smart Camera makes high speed and intelligent image processing possible.

It needs to be seen to be truly appreciated. The ball drops. It picks up speed during a free fall. Then three pincers grab it with perfect timing. Even if the ball is off center, the pincers adjust, bouncing the ball from one to the other before securing it. The pincers know what to do.

The heart of this precision robotic system is Hamamatsu Photonics Smart Camera. Conventional cameras used in artificial vision only capture 30 frames per second. Hamamatsu Photonics Smart Camera uses a column parallel vision system that can capture and analyze images, and direct mechanical movement, in less than a millisecond.

The system is based on a parallel structure in which each pixel employs its own dedicated processor. The parallel system is streamlined into a compact controller board.

This system will enable faster and more precise tracking and analysis- a boon for applications in the automation of factory equipment, vehicle and robot control, and inspection, as well as microscopy, the tracking of eye movements, and the analysis of movement in athletics.

Hamamatsu Photonics Smart Camera is being developed in ongoing cooperation with the University of Tokyo, Ishikawa Namiki Komuro Laboratory.

Hamamatsu Photonics' expertise with light has revealed an elegant solution to a complex problem.

HAMAMATSU

Photon is Our Business

For more information, visit our site at: http://jp.hamamatsu.com/en/rd/publication

HAMAMATSU PHOTONICS K. K.
325-6, Sunayama-cho, Naka-ku, Hamamatsu City, 430-8587 Japan Telephone: (81)53-452-2141, Fax: (81)53-456-7889 URL http://www.hamamatsu.com